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Development of a Natural Rearing System to Improve Supplemental Fish Quality

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DEVELOPMENT OF A NATURAL REARING SYSTEM TO IMPROVE SUPPLEMENTAL FISH QUALITY

1996-1998

PROGRESS REPORT

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EXECUTIVE SUMMARY

This report covers the 1996-1998 Natural Rearing Enhancement System (NATURES) research for increasing hatchery salmon postrelease survival and producing fish with more wild-like behavior, physiology, and morphology prior to release. Experiments were conducted evaluating automatic subsurface feeders; natural diets; exercise systems; seminatural raceway habitat enriched with cover, structure, and substrate; and predator avoidance conditioning for hatchery salmonids. Automatic subsurface feed delivery systems did not affect chinook salmon depth distribution or vulnerability to avian predators. Live-food diets only marginally improved the ability of chinook salmon to capture prey in stream enclosures. A prototype exercise system that can be retrofitted to raceways was developed, however, initial testing indicated that severe amounts of exercise may increase inculture mortality. Rearing chinook salmon in seminatural raceway habitat with gravel substrate, woody debris structure, and overhead cover improved coloration and postrelease survival without impacting in-culture health or survival. Steelhead fry reared in enriched environments with structure, cover, and point source feeders dominated and outcompeted conventionally reared fish. Exposing chinook salmon to caged predators increased their postrelease survival. Chinook salmon showed an antipredator response to chemical stimuli from injured conspecifics and exhibited acquired predator recognition following exposure to paired predator-prey stimuli. The report also includes the 1997 Natural Rearing System Workshop proceedings.

NATURES type research is called for in the Columbia Basin Fish and Wildlife Program (sections 7.2D.1-3) and the ESA Snake River Salmon Recovery Plan (sections 4.4.c-d). NATURES can be used to help restore depleted stocks within the Columbia River Basin. Conservation programs may benefit from increased postrelease survival to produce more recruits to the spawning population. Production programs can use NATURES to reduce their impact on wild-reared fish by releasing fewer smolts to negatively interact with wild fish, while maintaining their recruitment levels to the fishery.

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PREFACE

Support for this research came from electricity rate payers of the Pacific Northwest through the Bonneville Power Administration.

DISCLAIMER

Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

INTRODUCTION

The National Marine Fisheries Service (NMFS) has been conducting Natural Rearing Enhancement System (NATURES) research since the early 1990s. NATURES studies have looked at a variety of mechanisms to enhance production of wild-like salmonids from hatcheries. The goal of NATURES research is to develop fish culture techniques that enable hatcheries to produce salmon with more wild-like characteristics and increased postrelease survival. The development of such techniques is called for in the Columbia Basin Fish and Wildlife Program. This document is the final report for the Supplemental Fish Quality Contract DE-AI79-91BP20651. Work underway from March 1999 and on will be reported under a new Bonneville Power Administration (BPA) NATURES contract (DE-99-AI-16750).

Over the history of the project, the effects of seminatural raceway habitats, automated underwater feeders, exercise current velocities, live food diets, and predator avoidance training have been investigated. The findings of these studies are reported here and in an earlier contract report (Maynard et al. 1996a). The first section of the current report summarizes the earlier research results. The remainder of the report focuses on research that has been conducted between 1996 and 1998. This includes studies on the effect of seminatural raceway habitat, automated underwater feeders, live food diets, predator avoidance training, and exercise on salmon and steelhead trout.

Traditionally, salmon (*Oncorhynchus* spp.) are reared in barren concrete raceways that lack natural substrate, in-stream structure, or overhead cover. The fish are fed in an unnatural manner with artificial feeds mechanically or hand broadcast across the water surface. This traditional approach has increased the egg-to-smolt survival of hatchery-reared fish by an order of magnitude over that experienced by wild-reared salmon. However, once hatchery-reared fish are released into the wild their smolt-to-adult survival is usually much lower than wild-reared salmon.

The reduced postrelease survival of hatchery-reared fish may stem from differences in their behavior and morphology compared to wild-reared salmon. After release, hatchery-reared fish are inefficient foragers and are often found with empty stomachs or stomachs filled with indigestible debris (Miller 1953, Hochachka 1961, Reimers 1963, Sosiak et al. 1979, Myers 1980, O'Grady 1983, Johnsen and Ugedal 1986). Their social behavior also differs, with hatchery-reared fish congregating at higher densities, being more aggressive, and displaying less territory fidelity than wild-reared fish (Fenderson et al. 1968, Bachman 1984, Swain and Riddell 1990). In the natural environment this results in hatchery-reared fish spending more time in high-risk aggressive behavior and less time in beneficial foraging behavior than their wild-reared counterparts. Hatchery-reared fish are also more surface oriented than wild-reared salmonids (Mason et al. 1967, Sosiak 1978). This increases their risk of being attacked by avian predators, such as kingfishers (*Ceryle* spp.), which search for fish near the surface. Although some of the differences between wild and hatchery-reared fish are innate (Reisenbichler and McIntyre 1977, Swain and Riddell 1990), many are conditioned and can be modified by altering the hatchery rearing environment. NATURES studies are aimed at developing a more natural salmon culture environment to prevent the development of these unnatural attributes in hatchery-reared fish.

The research conducted under NATURES all follows the same problem-solving approach. The basic method is to: 1) develop fish rearing protocols which may produce more wild-like salmon with increased postrelease survival, 2) evaluate each protocol on a pilot scale to determine if it produces wild-like salmon with increased in-stream survival, 3) refine and reevaluate the protocols, 4) select the best protocol, and 5) evaluate its effect on smolt-to-adult survival with production-scale releases. At every stage of the research the project incorporates the scientific method of strong inference for solving problems, compares experimental treatment fish with conventionally-reared controls, incorporates testable null hypotheses which can be refuted, and uses statistical analysis in decision making.

NATURES fish culture practices are already producing salmon with a 10-50% higher in-stream survival than conventionally-reared fish (Maynard et al. 1996b). When these techniques are incorporated into production releases, they should also translate into increased smolt-to-adult survival. Conservation and supplementation programs can use NATURES-reared salmonids to rebuild stocks currently listed as endangered and threatened into healthy self-sustaining runs more rapidly than traditional programs. Traditional production programs can also use high-survival NATURES-reared fish to reduce their impact on wild populations, while still meeting their adult mitigation goals.

The following is a list of NATURES related articles published during the project:

Berejikian, B.A., E.P. Tezak, S.C. Riley, and A.L. LaRae. In review. Social behavior and competitive ability of juvenile steelhead (Oncorhynchus mykiss) reared in enriched and conventional hatchery tanks and a stream environment. J. Fish Biol.

Berejikian, B.A., E.P. Tezak, A.L. LaRae, T.A. Flagg, E. Kummerow, and C.V.W. Mahnken. 2000. Social dominance, growth and habitat use of age-0 steelhead (*Oncorhynchus mykiss*) grown in enriched and conventional hatchery rearing environments. Can. J. Fish. Aquat. Sci. 57:628-636.

Berejikian, B., E. Tezak, T. Flagg, A. LaRae, E. Kummerow, and C. Mahnken. 2000. Competitive ability and habitat use of steelhead fry grown in conventional and enriched hatchery vessels. Proceedings of Aquaculture America 2000, New Orleans, LA, p. 24.

Berejikian, B.A., R.J.F. Smith, E.P. Tezak, S.L. Schroder, and C.M. Knudsen. 1999. Chemical alarm signals and complex hatchery rearing habitats affect anti-predator behaviour and survival of chinook salmon (*Oncorhynchus tshawytscha*) juveniles. Can. J. Fish. Aquat. Sci. 56:830-838.

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Flagg, T., D. Maynard, and L. Harrell. 1999. Health and survival of chinook salmon reared in structurally enriched NATURES habitats. Proceedings of the combined annual Western Fish Disease Workshop and American Fisheries Society Fish Health Section meeting, Twin Falls, ID, p. 3.

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Maynard, D., T. Flagg, C. Mahnken, C. Johnson, B. Smith, and R. Iwamoto. 2000. Seminatural raceway environments as a tool for increasing the postrelease survival of chinook salmon released from conservation hatcheries. Proceedings of Aquaculture America 2000, New Orleans, LA, p. 212.

Maynard, D.J., A.L. LaRae, G.C. McDowell, G.A. Snell, T.A. Flagg, and C.V.W. Mahnken. 1998. Predator avoidance training can increase post-release survival of chinook salmon. *In* R. Z. Smith (editor), Proceedings of the 48th Annual Pacific Northwest Fish Culture Conference, Dec. 2-4, 1997, Gleneden Beach, OR, p. 59-62.

Maynard, D.J., E.P. Tezak, M. Crewson, D.A. Frost, T.A. Flagg, S.L. Schroder, C. Johnson, and C.V.W. Mahnken. 1998. Seminatural raceway habitat increases chinook salmon post-release survival. *In* R. Z. Smith (editor), Proceedings of the 48th Annual Pacific Northwest Fish Culture Conference, Dec. 2-4, 1997, Gleneden Beach, OR, p. 81-91.

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Section 1

SUMMARY OF 1991-1995 RESEARCH[1]

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[1] Based on Maynard, D.J., T.A. Flagg, and C.V.W. Mahnken (editors). 1996. Development of a natural rearing system to improve supplemental fish quality, 1991-1995. Report to Bonneville Power Administration, Contract DE-AI79-91BP20651, 216 p.

In this section we provide a summary of research results from our 1991-1995 NATURES studies to provide background of research leading to the 1996-1998 studies described in this report. The 1991-1995 research was a collaboration between NMFS, BPA, the Washington State Department of Fish and Wildlife (WDFW), and the U.S. Fish and Wildlife Service (USFWS) to develop and evaluate innovative culture techniques to increase postrelease survival of hatchery fish.

The WDFW Planning and Research Group (Olympia, Washington) identified fish marking and tagging procedures suitable for NATURES. Identifying (i.e., marking) fish is an essential component of evaluating the effects of various NATURES rearing strategies. Marking methods best suited for NATURES studies are those which will not affect their behavior, growth, locomotion, or survival, and meet other general requirements such as long-term retention and readability.

Mutilation and external tags are not acceptable marking methods for NATURES studies because of their adverse effects on behavioral and physiological factors. Branding techniques have been used in experiments for several decades and can provide a long-lasting external mark. Recent advancements in laser technology have improved the potential for laser marking as a viable tool; this mark may be more benign than branding and may last through the lifetime of some fish, particularly if methods can be developed that mark the soft fin rays.

Visual implant (V.I.) tags also show promise for use in NATURES studies. Injection of fluorescent materials have the advantage of being invisible until revealed by remote interrogation, thus eliminating observer bias and interactions between fish that might be associated with an externally visible tag. Panjet marks can also have a long retention time (i.e., several years) and can be used to mark young (30-40 mm) salmon fry.

Perhaps the two greatest concerns regarding all marking techniques are their degree of underwater visibility and their influence on fish behavior. Preliminary field evaluations of various marks were undertaken in 1992 to help establish protocols for evaluating these concerns. These observations indicated that laser marks were not retained as long as V.I. tags or adipose clips, and that the visibility of V.I. marks depended on light intensity and location of the mark on the fish.

Unfortunately, branding, laser techniques, V.I. tags, and panjet marks require further research to determine their effects on physiology and fish behavior. Therefore, PIT tags were chosen for mark/recapture studies described in this report, since these tags allow nonintrusive identification of treatment fish at recapture weirs.

Several pilot investigations were undertaken prior to commencement of full-scale NATURES research.

The NMFS Newport Laboratory conducted laboratory research on the feasibility of conditioning salmonids to avoid predators. In these studies, coho salmon (*O. kisutch*) disturbed by physical stressors demonstrated higher blood cortisol levels and vulnerability to predation by lingcod (*Ophiodon elongatus*) than non-stressed fish. Spring chinook salmon that had prior exposure to predation were less vulnerable to predation when compared with those that had not been previously exposed. Antipredator conditioning and stress reduction appeared to be keys for ameliorating the negative impacts of hatchery rearing on postrelease survival for juvenile salmon.

Nevertheless, a subsequent experiment failed to demonstrate the efficacy of predator conditioning in improving postrelease in-stream survival of fall chinook salmon. For this study, fish were reared to age-0 smolts using standard fish culture techniques. Test groups were then allocated to one of two identical 2.2-m diameter circular tanks; the "training" tank held two predatory cutthroat trout (*O. clarki*) whereas the control tank had no predators. The fish were held under these conditions for 16 hours prior to release into a small coastal stream. This procedure was replicated six times. There was no significant difference in the proportion of trained and untrained smolts recovered at a downstream weir. It is possible that antipredator training procedures used in this study were not extensive enough to improve antipredator recognition or antipredation responses.

The USFWS Abernathy Salmon Culture Technology Center reviewed information regarding feeds and feed delivery systems designed to reduce stress in hatchery fish. Factors controlling feeding behavior of wild salmon include vision, olfaction, taste, diet and seasonal feeding patterns, and prey characteristics. All of these factors must be addressed in developing a new generation of hatchery fish food, and this can be done by use of live feeds and/or developing artificial feeds with diverse shapes, textures, colors, and scents that elicit wild-like feeding responses in the fish.

Feed extrusion technology offers the ability to produce commercial feeds with wild food attributes. For instance, long, thin pellets can be produced, which have been shown to elicit stronger feeding responses than standard pellet shapes. Ideally, feed should be delivered below the surface in a drift form with enough current to keep it in suspension. Feed should also be delivered in low volumes, at high frequency, and at random subsurface locations throughout the raceway to simulate invertebrate drift patterns and to minimize territorial behavior and aggression in fish.

The NMFS Manchester Research Station initially investigated the use of live-food supplementation to increase the postrelease foraging ability of hatchery-reared fall chinook salmon. Replicate groups of fry were reared in six 2.4-m diameter circular tanks and fed on two different diets. Fish in three tanks received a standard, commercially available, pelletized diet, while those in the other tanks were given the opportunity to forage on natural live prey (mysids, mosquito larvae, chironomid larvae, and daphnia) prior to their daily ration of pellets. When foraging ability of individual fish was examined in 200-L observation tanks, the trained salmon were found to feed on twice the number of familiar prey (chironomids) and novel prey (mayfly larvae) as untrained fish. This work suggested that live-food supplementation could be used to increase the postrelease foraging ability of hatchery-reared salmon.

Nevertheless, a subsequent experiment failed to demonstrate the efficacy of live-food supplementation in improving in-stream foraging efficiency of spring chinook salmon. In this experiment, 24 replicate groups of yearling fish were held in 400-L tanks for approximately the last 60 days of rearing. The fish in all tanks received an equal volume of feed pellets each day. Fish in 12 tanks were given an additional ration of brine shrimp or tubifex worms prior to being fed pellets. At the end of the rearing period, the foraging efficiency of groups of test and control fish was evaluated in both freshwater and marine test arenas by allowing the fish to forage on natural prey for about 1 week.

Comparison of stomach contents from fish in the experiments indicated no significant difference in trained and untrained fish. Given observations of successful forage training with other species, it is surprising that habitat enrichment in this study had no effect on postrelease foraging ability. However, this observation may have been the result of very few fish in the study feeding, since many fish had little digestible material in their stomachs and most did not appear to have been feeding as well as they should. For habitat enrichment to enhance foraging ability, it may be necessary to instill a preference for live food diets earlier in the rearing cycle of salmonids.

The NMFS Manchester Research Station evaluated the effectiveness of various components of NATURES habitat concepts in three postrelease survival experiments conducted on chinook salmon. In the first experiment, fall chinook salmon were reared for 4 months from swim-up to smoltification. These fish, which were cultured in 400-L raceways outfitted with cover, structure, and substrate, survived in-stream travel to a collection weir 2.2 km downstream at a rate 50% higher than conventionally-reared salmon. In the second experiment, spring chinook salmon were reared for 3 months in 400-L raceways outfitted with cover, structure, and substrate. In clear water, these fish survived at a rate 24% higher than controls after release and traveling 225 km downstream to a collection weir. However, when fish were released in turbid water conditions, there was no significant difference in postrelease survival between test fish and controls.

In the final experiment conducted in conjunction with WDFW, culture vessel size was increased to 5,947 L, and fall chinook salmon were reared for about 4 months from swim-up to smoltification. NATURES raceways were outfitted with similar types of cover, structure, and substrate used in the other two experiments. However, in this study, an underwater feed delivery system was added to the NATURES treatment. In this experiment, NATURES fish averaged 27% higher postrelease survival to a collection weir 21 km downstream than their conventionally-reared counterparts.

In these studies, the NATURES variables tested succeeded in producing more “wild-like” fish than conventional rearing models. NATURES fish developed light and dark mottled body camouflage coloration patterns that were cryptic for the diverse stream bottom background over which these fish were released. In contrast, the uniformly light colored, conventionally-reared fish were cryptically mismatched for their release environment and required over 1 week of stream residence to begin development of the long-term color adaptation that can provide cryptic camouflage coloration for the stream background. By our subjective observations, the NATURES fish also displayed a greater fright response to overhead movement than the conventionally-reared groups.

The high prerelease survival (98%+) of both conventionally- and NATURES-reared fish in all studies suggested that the NATURES culture techniques we tested did not adversely affect fish health.

The 25-50% survival advantage during migration in the stream corridor for most groups of NATURES fish observed in the 1991-1995 studies was primarily due to the external camouflage color patterns of NATURES fish, which probably reduced their susceptibility to predation by visually hunting predators (e.g., birds and other fish). This may be why survival advantages were not noted for NATURES fish released in turbid water conditions where relative visibility was reduced. However, in the last experiment, it is probable that the automated underwater feeding system also lessened predator vulnerability of NATURES fish by inducing benthic orientation.

Our research demonstrated that rearing-habitat modification techniques developed in pilot-scale NATURES studies could be implemented in production fish rearing. We demonstrated that modification of the culture environment can produce significant positive differences in behavior and postrelease survival of hatchery fish in streams. The research demonstrated that rearing chinook salmon in NATURES environments with substrate, in-stream structure, and overhead cover increased in-stream postrelease survival. Our research also suggested that providing feed in the water column instead of at the surface could enhance fish foraging behavior. Vacuuming substrates was the only NATURES raceway operation procedure requiring a significant increase in maintenance effort. This was an important step in developing NATURES culture habitats for producing “wild-like” fish from hatcheries for use in genetic conservation and supplementation programs.

Section 2

THE EFFECT OF AUTOMATED SUB-SURFACE FEEDERS ON THE BEHAVIOR AND PREDATOR VULNERABILITY OF FALL CHINOOK SALMON

by

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Introduction

Research on feed delivery systems usually focuses on their effects on feeding efficiency, growth, in-culture survival, and in-culture behavior (Alanara 1992, Cho 1992, Tidwell et al. 1991, Tipping et al. 1986). However, with anadromous species like chinook salmon, which often have less than a 2% postrelease survival, it is also crucial to determine how these systems affect their predator vulnerability.

Traditional surface feeding techniques, with pellets broadcast on the surface by hand or moving vehicle, may condition behaviors in salmonids which increase their vulnerability to predators after release. In natural streams, salmonids feed on small insects falling off terrestrial vegetation, emergent insects drifting in the water column, or aquatic insects crawling on the river bottom. More importantly, they maintain an innate wariness of large moving objects at the surface. When a new stimulus, such as the silhouette of a bird, enters their visual field they may respond by (i) orienting to the object and freezing, (ii) rapidly fleeing away from the object, or (iii) seeking cover. The response of hatchery-reared salmonids to large moving objects at the surface delivering food is starkly different. Instead of fleeing, hatchery fish rapidly become conditioned to swim to the surface and fearlessly approach people and vehicles. This conditioned response is displayed even when food is not being delivered. In theory, if this conditioned response were generalized to all moving stimuli at the water surface it would increase the predatory risk from avian predators after release. Similarly, if surface feeding conditions hatchery salmon to be more surface oriented than their naturally-reared counterparts, then it makes them highly vulnerable to avian predators.

Automated subsurface feed delivery systems may be a tool for fish culturists to prevent hatchery-reared salmon becoming conditioned to approach large moving objects at the surface. Such feeders should condition fish to become bottom oriented and maintain their innate wariness of surface objects.

This study compares the depth preference, fright response to models, and predator vulnerability of salmon reared on traditional hand surface feeding and a new automated subsurface feed delivery system. The information is then used to evaluate the automated subsurface feed delivery system for conditioning behaviors to make juveniles less vulnerable to predators after they are released from hatcheries. The results are also used to determine if behaviors conditioned by the traditional hand feeding technique are specific to the object delivering feed, or to any large moving objects at the surface in general.

Methods

The study was conducted with 28,800 swim-up fry of fall chinook salmon (Soos Creek stock) donated by the Soos Creek Hatchery, operated by WDFW. The fish were transported as swim-up fry to the Manchester Research Station in February 1996. They were maintained in two rearing troughs (4-m long) until the experiments commenced. During this pre-experimental period the feed was broadcast on the surface by automated belt feeders.

After two weeks the fish were assigned randomly into six equal lots, numbering 4,800 per lot, and ponded into six raceways (6.4 × 1.5 m, and 0.6-m water depth). The fish in three raceways were fed a standard commercial moist pellet diet through an experimental automated subsurface delivery system. Fish in the other three raceways acted as controls and were fed the same diet broadcast by hand on the surface. Except for the method of feeding, the fish in both treatments received identical husbandry following standard salmon culture protocols.

The automated feed delivery system evaluated in the study was a modification of the system used at the WDFW Bingham Creek Hatchery and described by Maynard et al. (1996). An Allen feeder was fitted into the top of a tapered fiberglass cone supplied with demand feeders (Fig. 1). The cone was supplied with water (40 L/min) pumped in just below the top of the taper. A pipe (2.54-cm diameter) carried water from the bottom of the cone into the raceway. The pipe was branched with fittings to produce four conjoined lines, which ran across the bottom of the raceway. The release of food from the Allen feeder was regulated by an electric timer. When the arms in the Allen feeder were triggered to spin, food dropped into the cone, where it was carried down the piping system and into the raceway by the flowing water. The ends of lines were bent slightly upwards so food would rise to the surface, simulating emergent insect larvae.

In May, the fish in each raceway were systematically sorted and about two-thirds of the fish from each raceway were released. The remaining one-third were anesthetized in tricaine methanesulfonate (MS-222) and tagged photonically for identification. In photonic tagging, a carbon dioxide powered injector is used to produce a high pressure jet that propels microspheres containing fluorescent dyes into the skin. The microsphere tags and injector were supplied by New West Technologies of Santa Rosa, California. Fish reared on the automated underwater feeder were tagged with invisible blue tags and those reared by surface broadcast with invisible red tags. The tagged fish were then returned to their respective raceways and reared as before.

The distribution of the fish in the raceways was evaluated from video observations made during rearing. On an observation day, a high-resolution monochrome video camera was submersed in each raceway and the behavior of the fish videotaped for 50 minutes. The camera faceplate was positioned (about 1.3 m) directly across from the center of a vertical grid (1.37 × 0.55-m deep). The grid was divided into four equal vertical sections and horizontally in the middle to create eight sections in total. Fish were not fed on the days when tapes were made and all six raceways were taped on the same day. The taping was repeated three times at intervals of one week. The tapes were analyzed using video-imaging software and the number of fish in each section was counted. The percentage of fish in each section was then computed and the percentages compared with a two-way ANOVA.

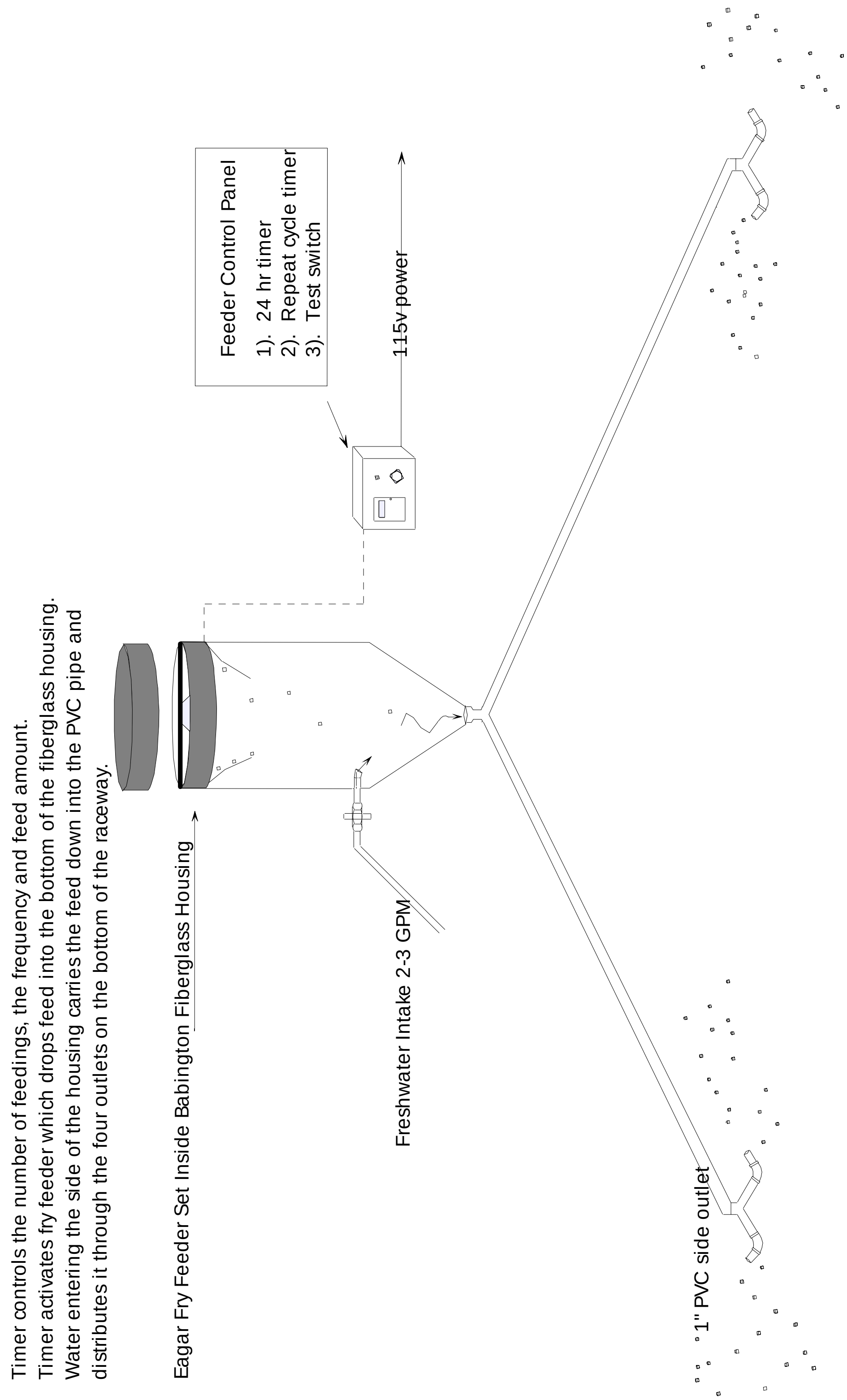

Figure 1. Automated subsurface feed delivery system.

In order to determine if the fish displayed the same depth preference in a new environment as in the tanks, their depth preferences were observed in laboratory aquaria (200-L capacity and 40-cm deep). Both sides and back of each aquarium were covered with gray PVC sheet. Horizontal lines (5.08-cm apart) were drawn across the back sheet to enable the observer to score the depth distribution of the fish. During each test the aquarium was illuminated from above, thus effectively hiding the observer.

A single test would consist of removing fish at random from each treatment, placing one fish in each aquarium, and acclimating them for 44 hours to their new environments. Two fish (one from each treatment) were observed during a trial which lasted 1 hour. The position of the fish in the tank was watched and scored at 10-minute intervals. The score (depth) for the six observations on each fish was averaged and the average depth distribution of fish in the two treatments was compared using a *t*-test. Twenty-one fish from each treatment were observed in the depth distribution tanks.

The predator vulnerability of hand- and automatic-fed fish was compared with bioassays conducted in the predation test arena at Manchester Research Station. The test arena consisted of rectangular tanks (6,000 L, $6.4 \times 1.5 \times 0.6$-m water depth) placed side by side and protected in a fenced area. A chain-link door separated the raceways from the holding area where hooded mergansers were held when not being used in bioassays. The bottom of each tank was lined with pea gravel. Algae were allowed to grow naturally on the sides of the tank and the entire arena was covered with a camouflage net to simulate a more natural environment.

In the first series of predation bioassays, 10 fish were selected from each rearing raceway and then transferred to one of the predation bioassay test arenas. Each tank was stocked with a total of 30 fish, with each tank receiving fish from only one rearing treatment. The fish were left in the arena overnight to recover from the effects of handling before mergansers were introduced the next day. After the birds had fished for three hours they were removed from the test arena, the tanks were drained, and the number of fish surviving was counted. This test procedure was repeated 29 times, with treatments alternated between the two tanks. Survival in the two rearing treatments was compared with a *t*-test.

The second series of bioassays was conducted similarly except fish from both rearing treatments were combined in each predation bioassay test arena. When these trials were initiated, fish were only available from two experimental and two control raceways. Five matched-length fish were removed from each raceway and transferred to one of the bioassay tanks. Each bioassay tank was stocked with 10 fish from each rearing treatment. The fish were acclimated overnight to recover from the effects of handling before exposure to the mergansers. The birds fished for only two hours due to the reduced number of fish. At the end of a trial the mergansers were removed from the arena, the water drawn down and the number of survivors from each treatment were identified. The relative survival of fish from the two rearing treatments was again compared with a paired *t*-test.

The response of fish to visual stimuli was observed prior to release. These trials were conducted by an observer located high (2.4 m) above the raceways recording responses to various visual stimuli. A second person moved along the outside of each raceway without being observed, ready to present one of the visual stimuli. The observer then told the carrier which stimulus to raise. The three types used were (i) a full scale model of a great blue heron, (ii) a pointed shovel, and (iii) the person him/herself. The trials were conducted with the stimuli until the response of the fish in all six raceways was recorded for each one. The treatment responses were then statistically compared with a Fisher's exact test.

Results

The behavior of the fish was observed each day from the beginning of the experiment. It was clear from the first day that fish in the two rearing treatments were displaying different responses to the approach of people. Fish in the automatic feeder treatment remained on the bottom and moved away from people approaching the raceway while fish in the hand-fed treatment rose up from the bottom and swam forward when people approached the raceway. This behavioral difference remained throughout the rest of the experiment.

Fish growth was monitored from the time they were initially placed in the experiment. When transferred from Soos Creek Hatchery, the fish averaged about 0.45 g and were about 38-mm long. Twenty-six days into the experiment (26 March 1996) fish in the automatic feeder treatment weighed 0.572 g and were 40-mm long, while fish in the hand-fed treatment weighed 0.593 g and were 39-mm long. During this first sampling period no significant differences were observed in fish weight ($P = 0.378$) or length ($P = 0.106$). On 18 April 1996, fish in the automatic feeder treatment averaged 0.679 g in weight and 42 mm in length and those in the hand-fed treatment raceways averaged 0.618 g in weight and 41 mm in length. Although not significantly ($P = 0.074$) different in weight, fish in the hand-fed treatment were significantly ($P = 0.045$) shorter than fish fed by automatic feeders. The overall poor growth of fish in both treatments was due to an infestation of *Costia*, diagnosed on 19 April 1996. The fish were successfully treated with a 1:6,000 formaldehyde bath on 20 April 1996.

A *t*-test indicated in-culture mortality was significantly ($P = 0.005$) higher for fish in the automated than hand-fed treatment (Fig. 2). The mortalities were primarily associated with an epidemic of *Costia*. The number of mortalities dropped sharply after formalin treatment and fish grew well thereafter.

A two-way ANOVA of the in-raceway depth distribution data indicated that, within both treatments, a significantly ($P < 0.001$) greater percentage of the fish were associated with the lower quadrants (Fig. 3). However, between treatments the depth distribution of the fish was nearly identical and not significantly ($P = 0.828$) different. Thus, chinook salmon in both treatments primarily resided in the lower half of the water column when the image of a person was not present within their visual field.

The laboratory observations of chinook salmon also demonstrated their innate benthic orientation, as fish from both treatments remained about 3.5 cm from the bottom. There was again no significant ($P = 0.883$) difference between the treatments, with fish from both rearing types displaying similar depth distributions in the laboratory test arenas (Fig. 4).

Fish from both treatments were equally vulnerable to merganser predation in both types of bioassays. When the treatments were placed side by side in separate raceways the fish were preyed on in nearly identical percentages, and there was no significant difference ($P = 0.722$) between treatments (Fig. 5). This was also the case when fish from both treatments were placed in the same raceway (Fig. 6).

The only distinct difference between fish from the two treatments was in their response to visual stimuli at the surface ($n = 3$). Presented with the model of a great blue heron or a shovel (novel stimuli), fish in both treatments, all six raceways, moved away from the stimulus and oriented themselves to keep it in view (Fig. 7a and b). When presented with the human image at the surface, the hand-fed fish in all three hand-fed raceways swam over to it, as if waiting to be fed, while the fish in all three automatic-fed raceways exhibited a strong fright response (Fig. 7c). Statistical analysis (one-tailed Fisher exact test) indicated the two treatments' fright response to the human image significantly ($P = 0.05$) differed, while the two treatments' fright response to the model heron and shovel did not significantly ($P = 0.50$) differ.

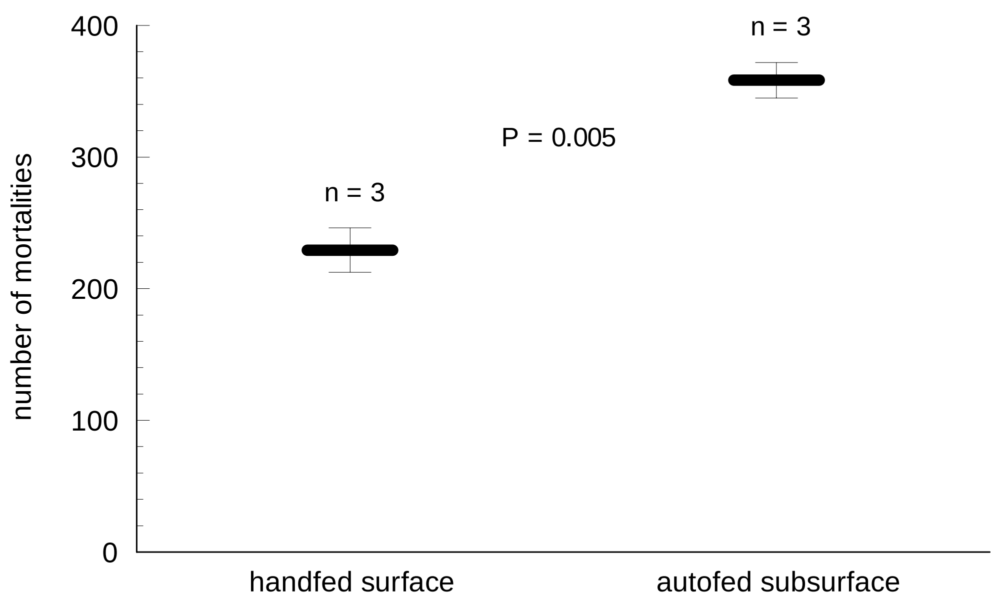

Figure 2. Average in-culture raceway mortality during 1996 underwater feeder study.

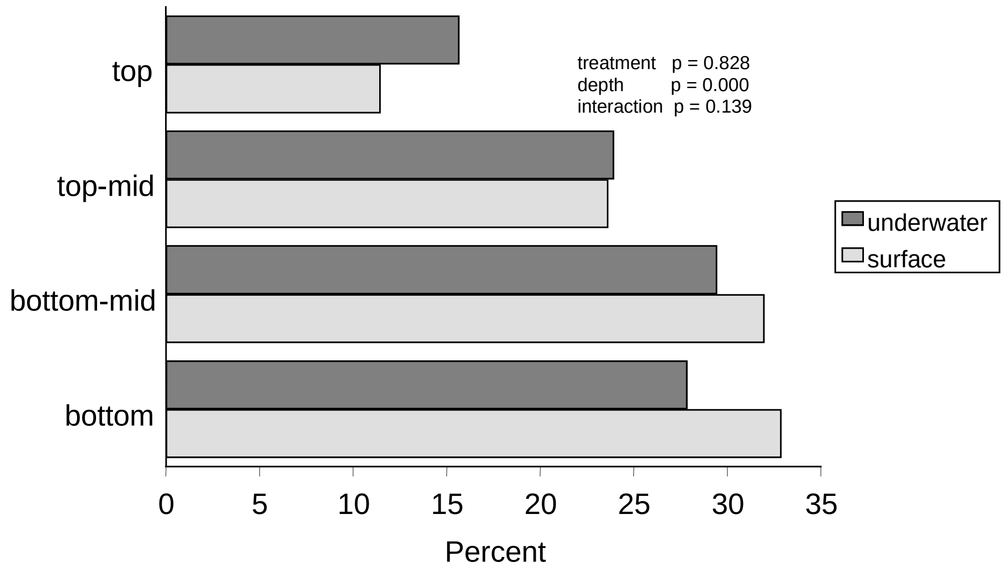

Figure 3. Average depth preference of fish videotaped in raceways.

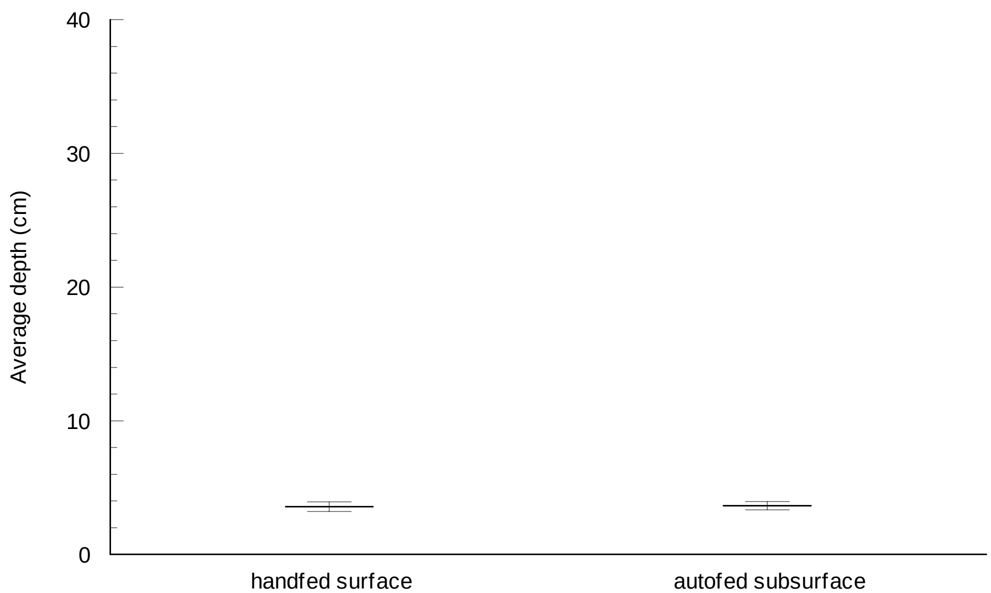

Figure 4. Average depth preference of fish in a laboratory test arena.

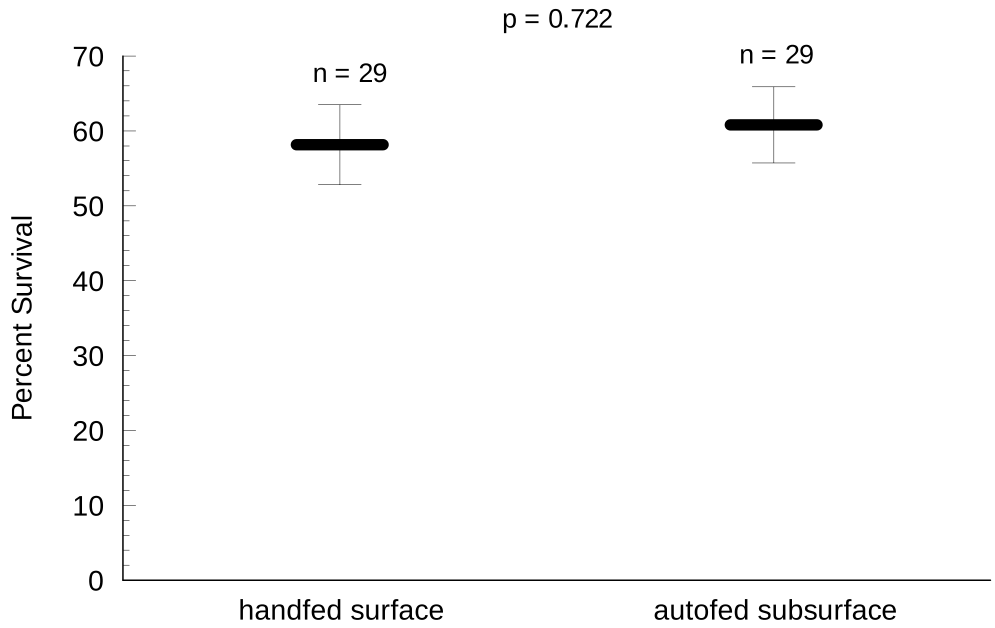

Figure 5. Percent survival after merganser predation with treatments in side by side test arenas.

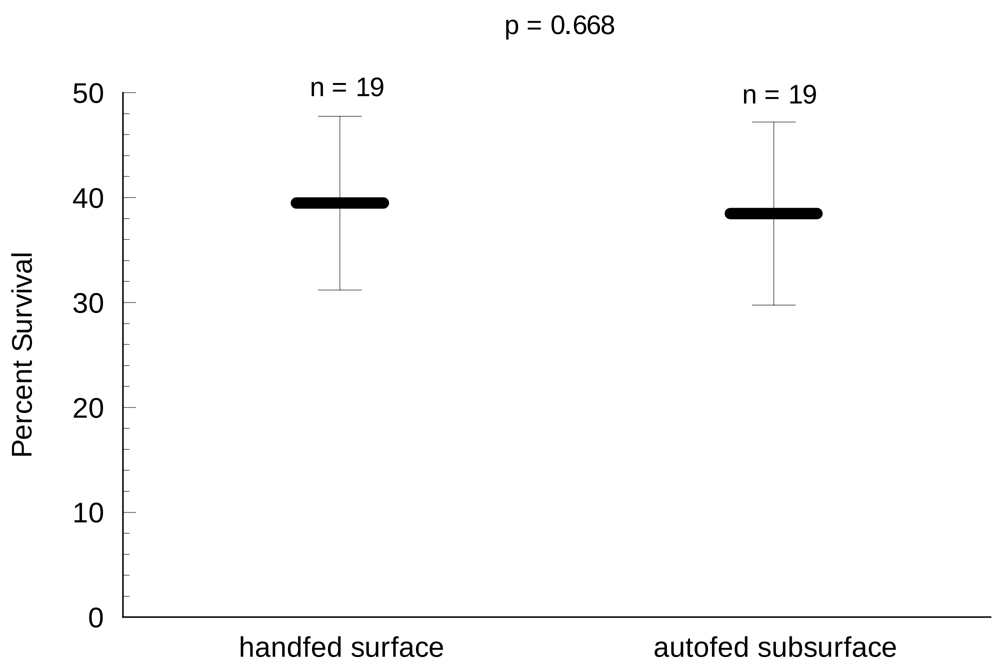

Figure 6. Percent survival after merganser predation in underwater feeder study with both treatments in the same test arena.

Discussion

The automated underwater feed delivery system failed to alter salmon behavior in a manner which might seemingly produce fish less vulnerable to predators. The hand-fed and automatic-fed fish exhibited identical depth preferences, the same response to unfamiliar objects at the surface, and similar vulnerability to predators. The only difference between the two groups was in their response to humans. The positive reward of food had conditioned hand-fed fish to swim towards humans when they were hungry. In contrast, the automatic-fed fish did not associate the human image with any reward. They continued to retain their instinctive response to freeze, remain at a distance, and orient towards any potential threat, whether it was a human being, a model of a heron, or a shovel entering their visual field.

The findings refute the premise that changing feeding methods will alter chinook salmon depth preference. The observations in the raceways and laboratory aquaria demonstrate that chinook salmon tend to reside at similar depths regardless of how they are fed. The aquaria data indicates that chinook salmon tend to remain near the bottom of the water column. These findings support the observations made in other studies (Dauble et al. 1989, Everest and Chapman 1972) that indicate chinook salmon have an innate preference to reside in the lower half of the water column. This does not preclude the fish from temporarily rising to the surface to feed, but they quickly return to deeper water after feeding. As the surface can be a dangerous place for a fish to reside, it is not surprising that hand feeding at the surface does not decondition the inherent tendency of these fish to remain away from it.

This study demonstrates that salmon have the ability to distinguish between specific visual stimuli such as a human image or a model of a heron. Under natural conditions, this specificity should permit fish to adapt gradually to all the neutral and positive visual stimuli they encounter while retaining their innate antipredator response to detect any negative and novel stimuli. This suggests that hand-fed salmon are at no greater risk of being preyed on than machine-fed fish when they are released into the natural environment. The only possible risk to hand-fed salmon would be human activity along the shoreline or in boats attracting fish to the surface where they then become vulnerable to predators. However, it seems unlikely that this conditioned response will generate any meaningful impact on the postrelease survival of hatchery fish.

The 5.2% mortality rate observed in the hand-surface fed treatment fish is similar to the 5.6% six year running average postponding mortality rate experienced by chinook salmon at the WDFW Soos Creek hatchery (Fuss and Ashbrook 1995). The 7.3% in-culture mortality experienced by fish reared on underwater feeders was a 40% increase over these base values. This may have been disease related. During feeding sessions, the underwater feeder may have attracted fish closer to the bottom where they became more vulnerable to parasites and pathogens or poor food hygiene may have contributed to the problem. Hand-fed fish generally received all their food soon after removal from storage, where it was always maintained in well-sealed and dry containers. Although the automatic-fed fish received their food equally promptly, invariably some food hung up in the feed delivery systems and this material became moldy and

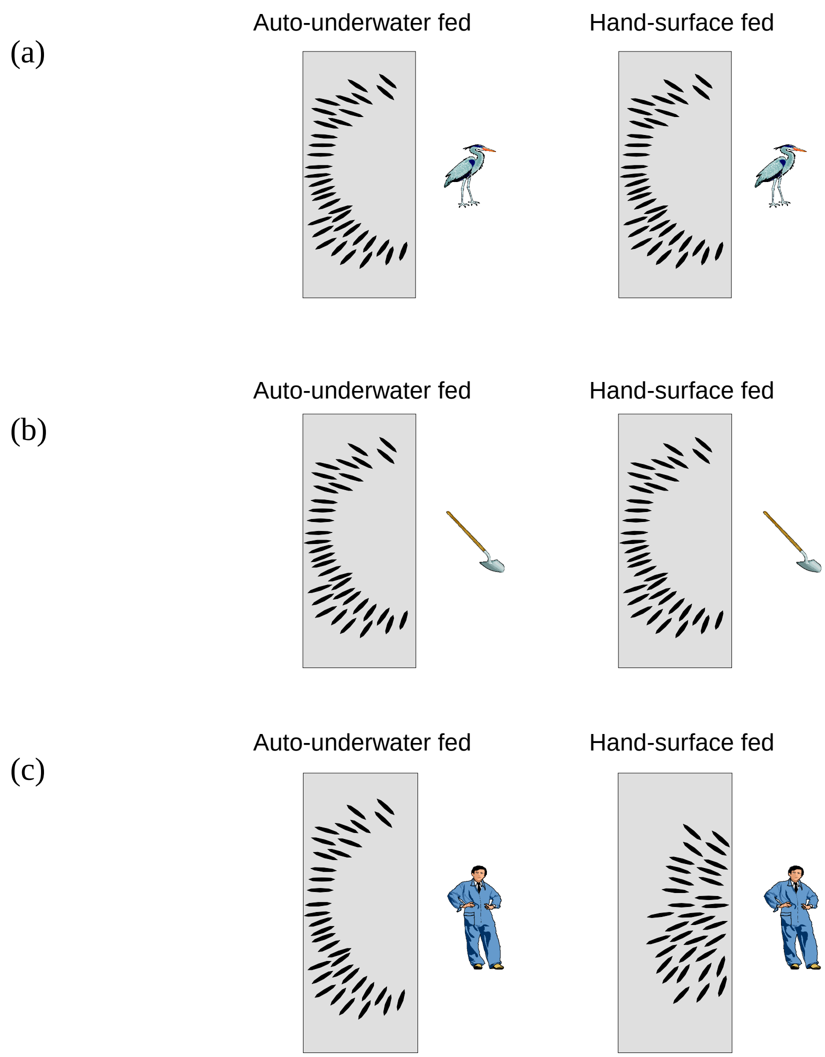

Figure 7. Salmon response to visual image of (a) great blue heron model, (b) shovel, and (c) human image.

decomposed. This material was removed during the weekly feeder cleaning, but may have been dislodged and fed to the fish prior to each cleaning. As research has shown that feeding moldy feeds can cause fish health problems (Ashley 1972, Stickney 1994, Roberts and Shepherd 1997) this aspect of the feed delivery system is probably responsible for producing the increased mortality observed in the automatic subsurface feeder treatment fish. It is recommended that in the future the feed delivery system be cleaned on a daily basis to avoid recurrence of this problem.

One difference the two feeding styles may induce is altered social competition. Scattering food across the surface by hand generally induces frenzied scramble competition in which dominant fish cannot successfully defend their food source (Thorpe et al. 1990, Grant 1993, Ryer and Olla 1996). In contrast, automatic subsurface feeders generate a point-source food supply which can be successfully defended. This scramble competition versus despotic competition was observed in an earlier study with a similar automated underwater feeder (Maynard et al. 1996). With steelhead trout, these point-source feed delivery systems seem to produce fish with increased social dominance (Berejikian et al. 2000). It therefore seems likely that a few fish receiving automated subsurface feed may become despots.

Hand-feeding fish is a traditional approach with several major benefits and no apparent drawbacks, except for being labor intensive (Goddard 1996). When fish are fed by hand their behavior and morphology can be observed, and used to detect the early appearance of disease or environmental problems in the population before they increase mortality. Hand feeding also ensures that feed is delivered fresh. Finally, with hand feeding it is easier to recognize the need to adjust the ration, as fish stop feeding when satiated or continue to search for food if they are still hungry. Observing a satiation response is important, as it not only prevents overfeeding but is also an indication that fish may have been lost due to undetected predation or screen failures.

In conclusion, the subsurface feed delivery system delivered feed well and should require little or no modification. In order to reduce in-culture mortality the entire system should be cleaned each day before the hopper is loaded. However, given the feeding system’s inability to change fish depth preference, fish response to novel stimuli, or produce fish less vulnerable to predators there may be no advantage in using an underwater feed delivery system. The only advantage of the subsurface feed delivery system may be when there is a need to produce fish with enhanced social dominance. Therefore, it is recommended that fish continue to be fed by hand because of the benefits associated with observing their feed response.

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Section 3

EFFECT OF LIVE FOOD DIETS ON THE FORAGING BEHAVIOR OF CULTURED FALL CHINOOK SALMON

by

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Introduction

Hatchery-reared salmonids may experience a low postrelease survival because they lack experience in foraging on natural prey (Miller 1952, Hochachka 1961, Reimers 1963). Typically, during the first few weeks after release, cultured salmonids eat less and forage on more indigestible material than wild-reared fish (Sosiak et al. 1979, Myers 1980, O'Grady 1983, Johnsen and Ugedal 1986, Hvidsten 1994, Fjellheim et al. 1995).

Several hypotheses may explain reduced foraging success and starvation; for example:

(i) newly released fish delay feeding until they are fully acclimated to their new surroundings;

(ii) pellet-reared fish are unable to recognize natural prey or find natural prey unpalatable as they could not develop a taste preference for it earlier in life;

(iii) pellet-reared fish must first develop experience in recognizing, capturing, and handling live prey which is considerably more cryptic and evasive than pellets before they can forage efficiently in their natural environment;

(iv) hatchery-reared fish may remain in denser aggregations than the prey base they encounter during their seaward migration can support.

However, it is probable that some combination of all these factors working together is the real reason why hatchery-reared fish appear to be starving when they are recaptured shortly after release.

This section describes experiments to determine if the use of live food diets improves the postrelease foraging ability of hatchery-reared salmon. The hypothesis is that live food diets will provide juvenile salmon with the experience they require to recognize, capture, handle, and ingest the natural live prey on which they will depend after release. Previous work (Maynard et al. 1996a) demonstrated that supplementing pellet diets with live feeds improved the laboratory foraging ability of fall chinook salmon. Using this information, the current study examines how a total live food diet affects both the laboratory and in situ foraging ability of fall chinook salmon.

Methods

In February 1996, six clear acrylic tanks (400-L capacity) were set up in an indoor fish culture laboratory at the Manchester Research Station. Each rectangular tank (152 × 46 × 46 cm) was supplied with a low flow (0.1-0.5 L/min) of city well water. The water in each tank was reprocessed by an aquarium filter (25 L/min) equipped with activated carbon, polyester fiber filter material, and a rotational wheel biofilter. Three sides of each tank were screened with gray PVC plastic sheet to prevent each group of fish observing the feeding behavior of others.

In March 1996, 300 fall chinook salmon swim-up fry were obtained from the WDFW Bingham Creek Hatchery. The fish were transported to Manchester Research Station and systematically divided into six equal lots of 50 fish. Each lot was ponded into one of the six tanks. The fish in three tanks were designated as controls and were reared exclusively on a commercial diet of semi-moist pellets. The fish in the other three tanks were fed the experimental live food diet of blackworms (*Lumbricules* spp.), brine shrimp (*Artemia salina*), bloodworms (*Chironomid* spp.), glassworms (*Coretrum* spp.), amphipod (*Gammarus* spp.) and shrimp (*Caridea* spp.). On rare occasions, when the required quantity of live food was unavailable, the diet was supplemented with whole frozen brine shrimp and krill (*Euphausiid* spp.). At first, the control ration was restricted so that the fish grew at a rate in parallel with the experimental fish. However, by October the control fish were visibly larger than those fed the live food diet.

The six groups were maintained in the tanks for six months. At the end of October 1996, they were transferred outdoors to six green circular fiberglass tanks (1.47-m diameter). Each tank was supplied with unfiltered well water (3.8 L/min). The subsequent handling and husbandry of fish in all tanks was similar and, apart from diets, followed standard salmon culture protocols.

The foraging behavior of fish from the two treatments was compared in rectangular tanks (400 L) identical to those described above. Three sides of each tank were totally covered and a black polyethylene curtain hung across the front, which could be opened to enable the behavior to be observed during each test. A layer of pea gravel (4 cm) covered the bottom of each arena to create a more natural foraging environment in which prey could hide.

Thirty-six laboratory foraging trials (eighteen per treatment) were conducted in the laboratory test arenas in September 1996. In each trial, a single fish was acclimated to the test arena for 48 hours before any prey were added. During acclimation the entire tank was covered, leaving the fish in total darkness. Two hours before each trial, the top cover was removed and a light turned on to allow the fish to adjust to the visual environment in which they would be observed. Fifteen minutes before the trail, prey was introduced into the tank through a gray PVC feeding tube (10-cm diameter). This gave the prey time to settle into the gravel so that fish were challenged both to recognize their prey and to seek it actively among the small stones.

The prey in each trial consisted of 20 amphipods, 20 blackworms, and 5 glassworms. The amphipods and blackworms were obtained commercially. The glassworms were collected from a small pond near the Manchester Research Station.

The treatments were alternated between trials until eighteen fish from each treatment were tested. Each trial was initiated by uncovering the front of the tank and removing the feeding tube. The frequency and time relationship for each prey the fish approached, attacked, captured, ingested, lost, or rejected, were recorded by an observer with the help of event-recorder software on a computer. The trial period lasted 60 minutes, after which the light was turned down, leaving the fish to forage overnight.

The prey handling time, which is a measure of the time taken by a fish to manipulate each item it ingests, was determined by observing an ingestion and tracing back through the record to find the original attack on that particular prey item. With this approach a fish capturing, rejecting, recapturing, and then ingesting a prey item was considered a complete string of events. Frequency of behaviors (approach, attack, capture, reject, and ingest) were analyzed with nonparametric Mann-Whitney U-tests. The prey handling time data were analyzed with *t*-tests.

In May 1997, after an additional 8 months of rearing, the foraging tests were initiated in situ. The fish were trucked in tanks, still separating each rearing group, from the Manchester Research Station to a location on Bingham Creek near the Bingham Creek Hatchery. They were placed in Bingham Creek, upstream of the hatchery, in twelve nylon net cages ($1 \times 1 \times 0.3$ m deep).

A single fish was placed in each cage, with six cages randomly receiving fish from one treatment and six from the other treatment. The cages were then submerged in the creek at least 2 m away from each other to prevent any interaction between fish. The only food available to these fish was natural prey in the creek that drifted, swam, or crawled into the cage. Both aquatic insects and salmonid fry were known to enter these cages.

After seven days the fish were retrieved and sacrificed. Their stomachs and intestines were then removed and preserved separately in formaldehyde solution (10%). The contents were weighed to the nearest 0.001 g. As it was difficult to distinguish reliably between digestible and indigestible material, only the weight of the total gut contents was used for data analysis. The gut contents are expressed as a percentage of overall body weight to compensate for differences in fish length. These foraging bioassays in situ were repeated over a five-week period until at least 25 fish from each treatment had been tested in the cages.

Results

Laboratory Trials

Prey behavior was the principal factor affecting fish foraging success. The blackworms immediately disappeared into the substrate. Consequently, the fish were never observed to approach, attack, capture, or ingest blackworms. Like blackworms, amphipods spent most of their time in the gravel where they were unavailable to the fish. Fish only detected and captured amphipods during the infrequent times they emerged and swam over the gravel surface for a short distance before burrowing again. During these movements six fish saw and ingested a total of 9 amphipods.

Glassworms, by contrast, stayed in the water column and were highly visible to the fish. This resulted in fish approaching, attacking, capturing, and ingesting glassworms more than any other live prey (Fig. 1). Thirteen fish from each treatment ate a total of 80 glassworms during the observation period. Fish spent most of their effort in approaching, attacking, and capturing non-food items, such as air bubbles and woody debris (Fig. 1). Nine fish actually ingested 12 non-food items.

No statistically significant difference was detected between treatments in the number of approaches, attacks, captures, or ingestions made towards any food item (Fig. 1). Fish from both treatments displayed similar behavior to live prey (Fig. 1a and b). However, pellet-fed fish were observed to approach, attack, and capture non-food items more often than fish reared on live food (Fig. 1c).

Although fish from both treatments attacked non-food items more often than anything else, pellet-reared fish directed a significantly ($P = 0.016$) larger percentage of their attacks at non-food items (Fig. 2). Similarly, fish fed live food directed a significantly ($P = 0.006$) greater percentage of their attacks at glassworms. The percentage of attacks directed at amphipods and blackworms was similar for both groups.

Foraging efficiency (ingests/attacks) was high for glassworms, followed by amphipods, and lowest for non-food items (Fig. 3). There was no statistically significant difference between treatments in their foraging efficiency on amphipods or non-food items. However, pellet-fed fish foraged significantly ($P = 0.014$) more efficiently on glassworms than live food fed fish.

In general, prey handling time was longest for amphipods, intermediate for glassworms, and shortest for non-food items (Fig. 4). With the relatively small sample sizes ($n = 3$-13) and large variance associated with behavior, it was not possible to detect any statistically significant differences between treatments in average prey handling time. Based on the actual data, the live food fed fish spent slightly more time handling live food and the pellet-reared fish slightly more time handling non-food items. This is in contrast to foraging theory that would predict that prey handling time should decrease with experience (Hughes 1979, Hughes et al. 1992). However in an earlier study we also saw a greater, although not statistically different, handling time of live prey for chinook salmon that had previous experience handling live food (Maynard et al. 1996a). Therefore, this may be more an indication of interest in these food types rather than the time needed to handle them.

Although they had not fed for two days and some of the prey were easy and attractive targets, 6% of fish fed live diets and 22% of pellet-fed fish did not attack anything in the test arenas (prey or non-food items). Seventeen percent of live food fed fish and 28% of pellet-fed fish failed to attack any live prey items and ingested nothing during observation

The In Situ Trials

The guts of many of the fish in the cages were near empty, with roughly 38.5% of the fish having a gut content that weighed less than 0.1 g (Fig. 5). Only 26.9% of the study fish (6 live food and 8 pellet-fed) had a gut content that weighed between 1 and 4% of their body weight. Only two fish had more than 1 g of material in their gut. While the guts of all of the live food treatment fish had some material inside, the guts of 3 of the pellet-fed fish contained nothing.

On average, the live food fed fish placed in cages were smaller than the pellet-fed fish (Fig. 6). The gut content weight of fish reared on a live food diet was greater than fish reared on a pellet-only diet (Fig. 7). However, with the high variance and small sample size, it was not possible to detect a statistically significant (P = 0.606) difference between the two treatments.

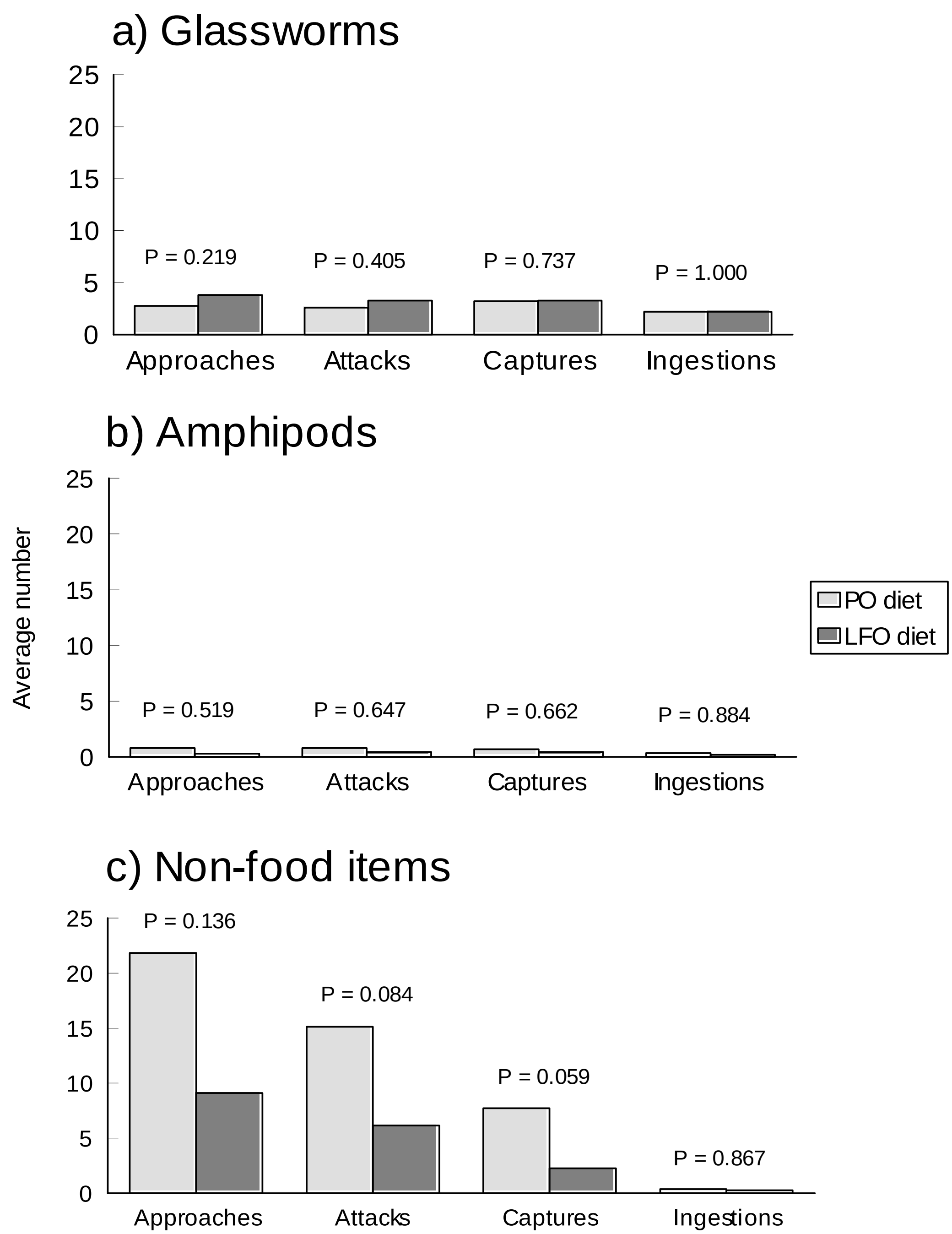

Figure 1. Foraging behavior of fall chinook salmon observed with (a) glassworms, (b) amphipods, and (c) non-food items. Salmon were reared on a pellet-only diet (PO; N = 18) or a live food-only diet (LFO; N = 18). The probability values (P) are based on Mann-Whitney U-tests.

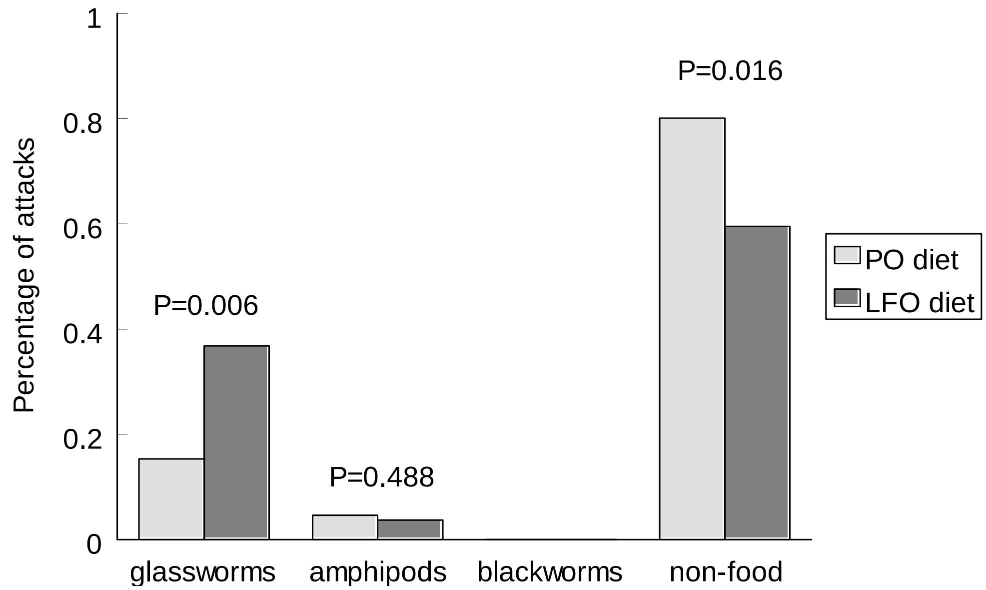

Figure 2. Percentage of attacks by fall chinook salmon on each prey item. Salmon were reared on a pellet-only diet (PO; N = 18) or a live food-only diet (LFO; N = 18). The probability values (P) are based on Mann-Whitney U-tests.

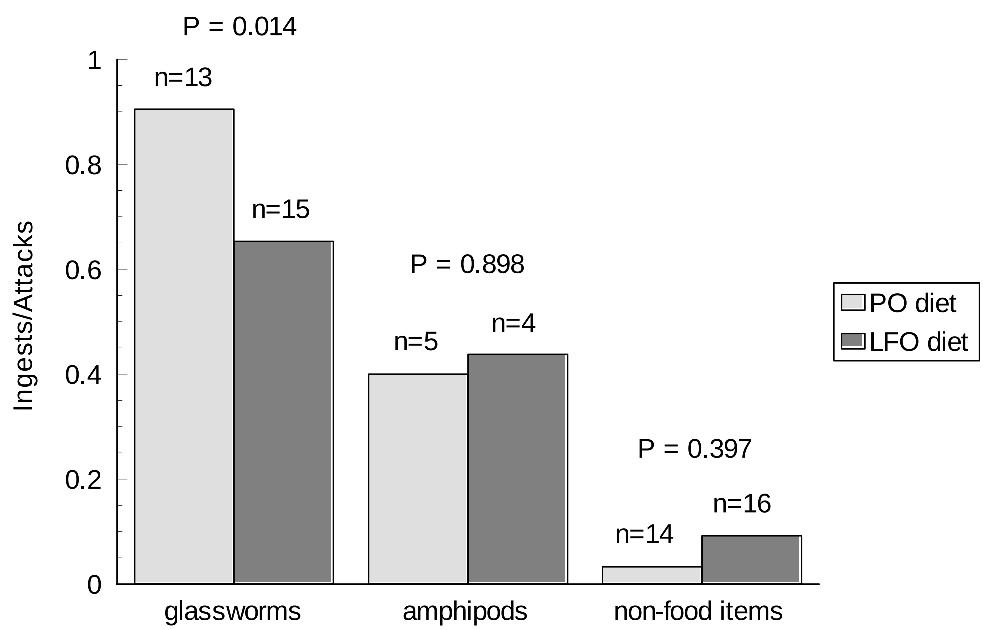

Figure 3. Foraging efficiency (ingests/attacks) of fall chinook salmon on each prey item. Salmon were reared on a pellet-only diet (PO) or a live food-only diet (LFO). Probability values are based on *t*-tests.

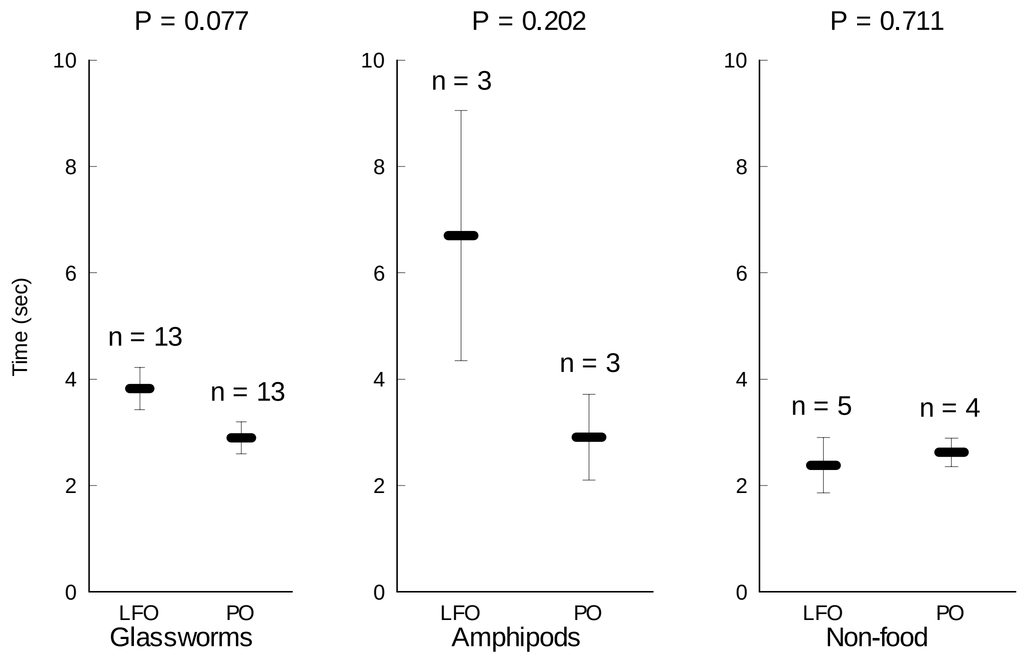

Figure 4. Average prey handling time of fall chinook salmon. Salmon were reared on a pellet-only diet (PO) or a live food-only diet (LFO). Probability values are based on *t*-tests.

Discussion

In the freshwater and estuarine environments juvenile fall chinook salmon feed primarily on insects, amphipods, shrimp (*Neomysis* and *Mysis* spp.), and zooplankton (Sasaki 1966, Busby and Barnhart 1995, Rondorf et al. 1990). Some time after they enter the ocean they switch over to primarily feeding on fishes (Brodeur 1991). In all three environments the prey are taken as they are observed drifting or swimming in the water column. As with most other predators, the prey of chinook salmon have evolved successful strategies to avoid being preyed on. Thus, in the laboratory, we observed that the antipredator characteristics of the prey had the strongest effect on salmon foraging success. As with other predators (Irvine and Northcote 1983, Sih and Moore 1990, Sih 1993), prey activity and movement were key factors in determining the prey chinook salmon selected to attack.

Blackworms successfully avoided predation by burrowing into the substrate. For the most part, the amphipods also successfully hid in the substrate and only became vulnerable to chinook salmon predation when they occasionally emerged and swam in the open water column. The glassworms were from a population lacking fish and appeared to not have evolved an effective strategy for avoiding fish predation. Their high visibility and swimming about in open water made them attractive targets for chinook salmon. This resulted in their being the most frequently consumed prey item in the test arenas.

Interestingly, fish from neither rearing treatment searched for blackworms or amphipods by rooting around in the gravel as if following a scent trail. As the fish in the live food diet treatment had extensive opportunity to learn blackworm scent prior to testing, it appears that chinook salmon do not search for prey based on chemical cues. This supports the concept that chinook salmon, like other salmonids, are primarily visual hunters that are initially attracted to prey based on their visual cues (Chapman and Bjornn 1969, Fausch 1991). Specifically, movement of the proper size and shape object within the visual field stimulates the initial approach, attack and capture of a potential prey item. Once the prey is captured, taste and texture then become the primary cues involved in making the decision to ingest or reject the captured prey item (Bres 1989, Willers 1991).

In the laboratory tests, the live food diet failed to improve chinook salmon foraging ability on both evasive (blackworm and amphipod) or easy to capture (glassworm) prey. Equally important, being reared on a pellet diet did not seem to preclude fish from being able to switch over to live food when it was readily available. However, the pellet diet did increase fish interest in attacking non-food items. In this regard, the greatest distinction between the two rearing types was the percentage of attacks salmon directed at each type of prey. The pellet diet fish directed a greater percentage of their attacks at non-food items than fish reared on live feed, while live diet fish directed a greater percentage of their attacks at glassworms than pellet-reared fish did. Although the results are not significantly different, the pellet-only fish actually performed more than twice as many attacks and captures on non-food items as live food diet salmon. Field studies have also observed that salmon reared on prepared pelletized diets initially tend to ingest more non-food plant material after release than fish reared on natural feeds (Johnsen and Ugedal 1986, Myers 1980). In combination, this laboratory and field evidence suggests that fish reared on pellets spend more of their activity budget in pursuing non-food items than salmonids reared on natural animal feeds.

The semi-moist diet the pellet diet fish were reared on in this study contains processed grain byproducts, lecithin, and guar gum from plants. In addition, the proximate composition of this artificial feed (43% protein, 14% crude fat, 2% crude fiber, 10.5 % ash, 22% moisture, and 1% phosphorous) markedly differs from the proximate composition of live animals, like chironomids (9.1% crude protein, 13.6% crude fat, 0% crude fiber, 0.9% ash, 83.9% moisture, 0% phosphorous), fed to the live diet fish (Cresswell 1993). These chemical and water content differences must result in pelleted feeds having a markedly different flavor and texture than the live animals that salmonids naturally consume. When salmonids are reared on artificial diets they must become conditioned to accept plant materials that they would otherwise reject. As a result of this deconditioning of the rejection response to vegetable material, pellet-reared salmonids must relearn to reject the vegetable debris they encounter in our test tanks, or the streams, rivers, or estuaries they initially reside in after release. In the learning process they will tend to spend more energy and time in pursuing this energetically unprofitable material. As natural prey are hard to find and estuaries retain large amounts of drifting vegetative debris, pellet-reared chinook salmon may starve to death before they learn which items to invest their time in attacking and capturing. Fortunately, the current trend in developing artificial diets is to remove plant fillers from feeds and replace them with ingredients derived from aquatic animals.

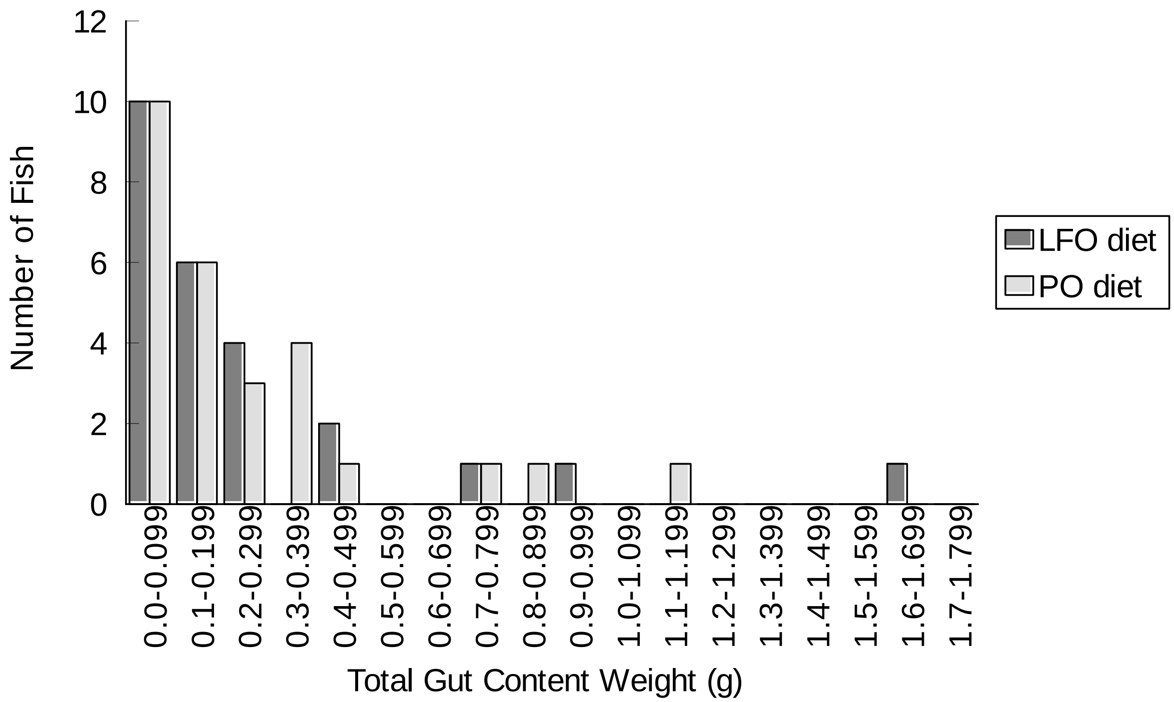

Figure 5. Histogram of total gut contents of fall chinook salmon placed in Bingham Creek cages. Salmon were reared on a pellet-only diet (PO) or a live food-only diet (LFO). Probability values are based on a Student *t*-test.

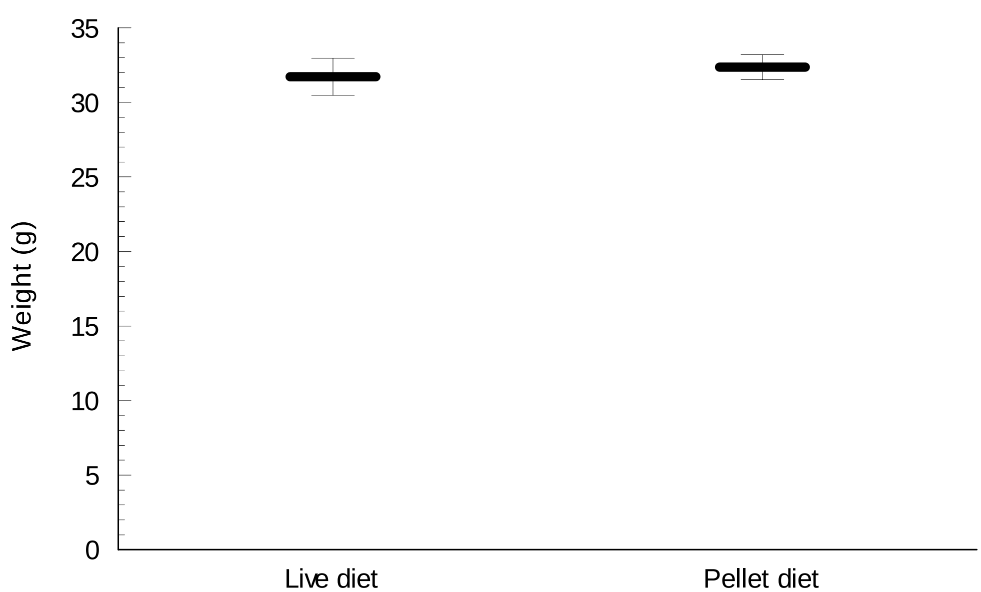

Figure 6. Average weight of fall chinook salmon placed in Bingham Creek cages.

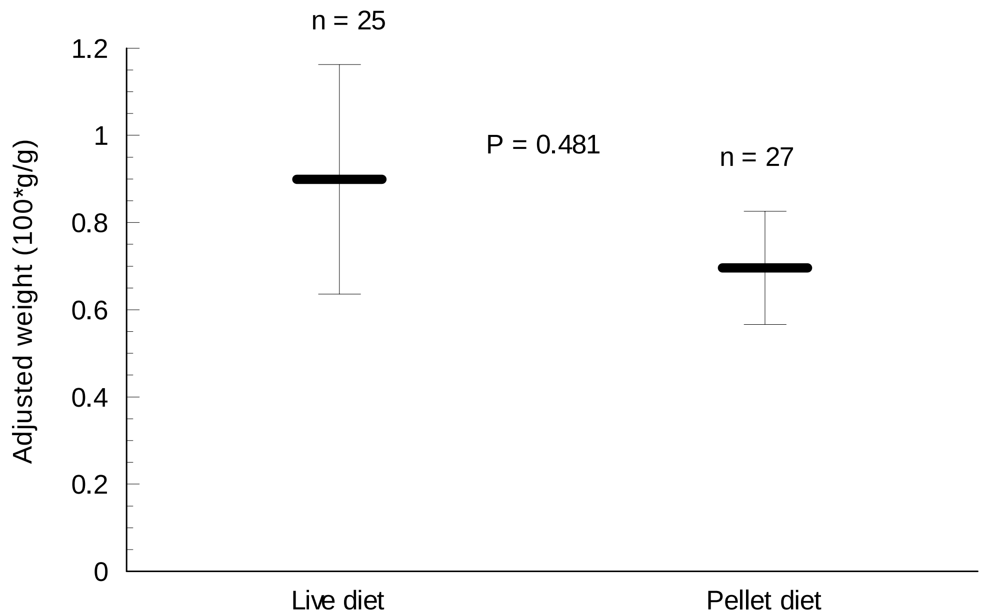

Figure 7. Percent body weight of material in gut of fall chinook salmon after residing in Bingham Creek cages for one week. Probability values are based on a Student *t*-test.

The stomach fullness of the fish from the in situ trials (cages) was considerably less than expected of fish feeding in the wild. The stomach contents of wild chinook salmon usually weigh more than 2% of their total body weight (Healey 1979). However, only 27% of the fish in this study even reached a 1% total gut (stomach and intestine) content. By the time the fish were tested in the enclosures, they averaged 32.06 g in weight, which would anticipate material in their gut to weigh over 0.321 g. However, 38.5% of the fish in the cages had less than 0.100 g in their gut. This lower than expected gut fullness of hatchery fish has been observed in an earlier study as well (Maynard et al. 1996b). In the earlier study it was suggested that social interaction may have precluded most of the fish in the cages from feeding. However that is clearly not the case in the current study where there was only one fish per cage. This lower than expected stomach fullness may be representative of a hatchery (rather than wild) fish model since other studies also indicate that it is common for hatchery fish released into the wild to initially have less material in their digestive system than expected (Sosiak et al. 1979, Myers 1980, O'Grady 1983, Johnsen and Ugedal 1986, Hvidsten 1994, Fjellheim et al. 1995).

Although not statistically significant, the increased amount of material in the guts of live food compared to pellet-reared fish suggests that live food diets might improve postrelease foraging ability. The presence of material in the guts of all live food diet fish, and only 88% of the pellet-reared fish, also suggests live food diets may improve postrelease foraging ability. These findings agree with other observations that the stomachs of hatchery-reared salmonids released into the wild often contain less food than naturally-reared fish (Sosiak et al. 1979, Johnson et al. 1996). It also agrees with an earlier finding with fall chinook salmon that supplementing artificial diets with live feeds can improve foraging behavior in a laboratory setting (Maynard et al. 1996a). However, at this time, the strength of these findings does not justify the expenses associated with rearing hatchery-produced salmon on a total live food diet.

The laboratory findings indicate that chinook salmon can learn to forage on new prey, like glassworms, when given the opportunity. This has been observed with other salmonid species as well. When initially exposed to three different prey types, hatchery-reared Atlantic salmon exhibited different prey preferences than wild-reared fish, however within a short time they switched over to match the prey preference of their wild-reared counterparts (Reiriz et al. 1998). In other studies, pellet-reared Atlantic salmon have also demonstrated their capability to learn to use new food types by switching over to wild prey (Stradmeyer and Thorpe 1987). It has also been shown that sockeye salmon can readily learn to forage more effectively on prey with repeated exposure to it. However, not all salmonids show this high level of prey switching flexibility. Hatchery-reared brook trout had difficulty in switching to alternate food items when they were made available (Ersbak and Haase 1983). Rainbow trout required four days of exposure to novel prey before they would even approach it, and another eight days before they demonstrated good prey capture responses (Ware 1971). Given fall chinook salmon seem to have the flexibility to switch over to new prey types, the question now becomes why don't they show better foraging success when they are challenged to forage naturally in field studies.

There may be explanations for the reason so many fish had little material in their guts. One possible explanation is that the fish, which like most salmonids are dusk and dawn feeders, simply digested most of the material before they were sampled at 1400 hours. This is unlikely as at 10^0 C the time to 50% evacuation of material in the stomach is 6 to 15 hours for salmonids (Brett and Higgs 1970, Windell et al. 1976). This would mean that even if fish had eaten the usual 2% of their body weight in the morning they should have at least 0.3g in their stomachs in the afternoon, rather than having a total gut content that usually weighed less than 0.2 g. Another explanation is that sufficient quantities of the right type of prey did not enter the cages. This appears unlikely, as various types of insect larvae were observed crawling on the inside of the cages when the fish were retrieved, but if they were noxious or otherwise predator resistant they may not have been useful food sources.

Possibly many fish were stressed by the new environment and not composed enough to forage for food. Other laboratory studies (Maynard et al. 1996a, Paszkowski and Olla 1985), and many field studies (Ersbak and Haase 1983, Maynard et al. 1996b, Miller 1952, Hochachka 1961, Reimers 1963, Sosiak et al. 1979, Myers 1980, O'Grady 1983, Johnsen and Ugedal 1986) have found that hatchery fish starve for several weeks after release. In the natural environment the fish are experiencing very different water sources, physical conditions (gravel, vegetation, overhead cover, temperature, sounds, and light levels), predators, and many other animals they never previously encountered. If this is true, then the problem might be overcome by acclimating the fish to natural environments prior to release, and using a good feeding response as the cue for adaptation to their new surroundings.

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Section 4

EFFECTS OF MODIFIED REARING ENVIRONMENTS ON THE VULNERABILITY OF JUVENILE CHINOOK SALMON (*O. TSHAWYTSCHA*) TO NATURAL PREDATORS

by

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Introduction

Seminatural rearing environments, that include a combination of overhead cover, in-water structure, gravel substrate, and subsurface feed delivery systems, can increase postrelease survival of smolts of chinook salmon compared with more conventional conditions for rearing (Maynard et al. 1996a, 1996b). The greatest disparity in postrelease survival occurred within the first 14 days following release, which suggested that, compared with other slower acting causes of mortality such as disease and starvation, differential predation accounted for most of the differences between the two groups. Salmon grown in semi-natural environments may benefit from morphological color changes (Fujii 1991), which improve cryptic coloration and reduce vulnerability to predation (Donnelly and Whoriskey 1993). However, there is no direct evidence that structurally modifying hatchery rearing environments affects the vulnerability of salmon to predators in nature.

The present research is part of a larger study conducted in cooperation with WDFW (principal investigators S.L. Schroder and C.M. Knudsen) to determine the effects of various structural modifications (i.e., seminatural habitats) on postrelease survival of smolts of chinook salmon. It is designed to test the null hypothesis that rearing juveniles in circular vessels modified with combinations of variables (such as, overhead cover, in-water structure, raised gravel substrate, and underwater feeders) will affect their vulnerability to predators. The vulnerability of smolts to predation is determined directly by exposing them to cutthroat trout in experimental raceways, and indirectly using a hypothetical surrogate measure of predation risk (i.e., incidence of predation marks) for smolts released into a natural stream. Predation risk is inferred by the incidence of injuries or marks on fish which were attacked or captured, but subsequently escaped (White 1936, Donnelly and Whoriskey 1993, Matity et al. 1994). In these studies, fish which had a high incidence of marks were presumed to be at high risk of predation.

Methods

Rearing Treatments

The effects of various rearing environments on postrelease survival (in the WDFW study) and predator avoidance ability (this study) of smolts of chinook salmon were studied in gray circular rearing vessels (1.8-m diameter). Seven rearing treatments were established:
(1) Control - a barren tank;
(2) Cover only - overhead cover of two layers of green and brown camouflage netting;
(3) Cover with substrate - overhead cover, and raised-bottom mesh substrate with scattered pea gravel;
(4) Cover with feeder - overhead cover and underwater feed delivery system;
(5) Cover with structure - overhead cover and in-water structure in the form of two rectangular PVC frames hung with a single layer of camouflage netting;
(6) Full complex - overhead cover, raised-bottom substrate, underwater feeder, and in-water structure, and

(7) Limited complex - with barren vessels later modified to a complex environment after 60 days of rearing, 30 days prior to release.

Twenty-eight equal-sized lots of 1,100 fry of chinook salmon were given a unique thermal code on their otoliths during incubation in Heath Trays (Volk et al. 1994). Each lot was stocked at the button-up stage of development into one of 28 vessels (4 vessels for each of the 7 treatments) on 1 March 1996. The fish were fed with a standard commercial salmon diet, four to five times a day and five days per week.

Incidence of Predation Marks

As part of the larger WDFW study, two mass releases were made to determine the relative in-stream postrelease survival of smolts cultured in each of the seven environments. The first release was made on 20 June 1996 and involved smolts from two of the four rearing vessels for each treatment (14 tanks in total) liberated into Bingham Creek at a location approximately 21 km upstream from a 100% efficient collection weir. The second, at the same site on 3 July 1996 involved fish from the remaining 14 tanks.

For this study, following their release in two groups, all the smolts were collected at the weir. Each fish was examined individually for external flesh wounds presumed to have been caused by predators. Marks were recorded by sketching observed wounds on form outlines of smolts, and classified as one of two main types:

(i) Multiple fine "rake" marks, often roughly parallel to one another, and occurring on both sides of the body. Sometimes, but not always, the skin was punctured. These marks are typical of piscine predators, or avian predators with serrated bills (e.g., the common merganser, *Mergus merganser*).

(ii) Blunt, V-shaped marks, with the point in a ventral direction. These marks are more consistent with diving birds (Smith and Lemly 1986), such as the kingfishers (*Ceryle alcyon*) which are numerous in Bingham Creek, and were observed feeding on salmon fry in in-stream cages (Berejikian et al. unpublished data).

Each smolt with a predator mark was individually labeled, preserved in ethanol, and later identified with its rearing environment by its otolith mark (Volk et al. 1994).

Vulnerability to Cutthroat Trout

Two adjacent concrete raceway channels at the WDFW Bingham Creek Hatchery were each divided by wire screens (6-mm mesh) into three sections (5 × 3 m). The bottom of each of the six sections was covered with a single layer of gravel (2.5-5 cm in diameter) to provide a natural substrate. Denuded Douglas fir trees (about 2 m in height) were placed along both sides of three of the sections to provide a natural refugia for the smolts; the other three sections had no refugia. Each raceway was filled to a depth of 45 cm with water from the East Fork Satsop River, and exchanged at a rate of approximately 400 L/minute.

Cutthroat trout were collected by hook-and-line from the East Fork Satsop River, approximately 200 m upstream from its confluence with Bingham Creek. The trout were first conditioned in two tanks (1.8-m diameter), starved for 1 week, and then fed juvenile chinook salmon once a week for 3 weeks. Five trout were then placed in each section on 1 June 1996 for acclimation, and fed with juveniles twice more (on 4 and 11 June 1996). Any remaining juveniles were removed on 17 June 1996, four days prior to the start of the first experiment.

Before each of the two releases of smolts into Bingham Creek, 30 smolts from each of the 28 tanks were removed. Those removed on 19 June before the first release were selected within the range 69-73 mm fork length. Those removed on 2 July before the second release were selected within the range 78-82 mm fork length. Five smolts from each treatment (two or three smolts from each of 2 tanks within a treatment) were stocked into each of the six cutthroat trout predation arenas on 21 June 1996 (round 1). After four days the predators were removed from each section and held temporarily in tanks while all the surviving smolts were removed by electrofishing. The trout were then returned to their respective sections and the procedure was repeated, beginning on 28 June with the remaining fish from release 1 (round 2). Two more sets of experiments were carried out on 4 July (round 3) and 13 July (round 4) with fish from the second release. In total there were twenty-four replicate trials, six carried out for each of the four rounds. The bodies of surviving juveniles from each trial were preserved in ethanol, and their otoliths decoded to identify their rearing treatments.

Vulnerability to Avian Predators

The vulnerability of the smolts to avian predators was tested for each of the seven rearing treatments. An additional 30 fish were sampled from each of the 28 tanks using the same procedures as described above. Five experimental cages were fabricated (2.7 × 1.3 × 1.3 m deep) and located within 2 km upstream from the confluence of East Fork Satsop River and Bingham Creek. Two were placed in Bingham Creek, two in East Fork Satsop River, and one in the outflow from Bingham Creek Hatchery. The bottom of each was covered with a layer of gravel from the surrounding streambed.

Six smolts from each treatment from the first release group were stocked in each cage on 21 June. An overhead count of survivors was made every other day to minimize the possibility of total loss. When the number in any cage had been reduced by about 30%, that cage was covered to eliminate further loss. After 5 days all smolts were removed by electrofishing. The procedure was repeated on 26 June with more smolts from the first release, and on 3 July and 10 July with smolts from the second release. Survivors were handled as before.

Statistical Analyses

A univariate linear regression analysis was made first to determine if the proportion of fish from each tank recaptured at the weir (independent variable) was significantly correlated with the proportion of fish with predator marks (dependent variable). A significant positive relationship (α = 0.05) between these two variables would validate the assumption that the incidence of predation marks could be used as an indicator of predation pressure.

The proportion of chinook salmon smolts from each treatment surviving four days of exposure to cutthroat trout was analyzed by a three factor ANOVA, with rearing treatment, raceway structure, and trial round (1 - 4) as the main effects. Because of the unequal, small number of fish taken from each rearing vessel for each trial (i.e., either two or three fish from each vessel per trial) the effects for individual tank was not tested. Tukey's HSD tests were used to test for differences among levels of a factor. Arcsine transformations were performed on proportion data generated from the experiment to improve normality.

Results

Incidence of Predation Marks

Of the 28,327 smolts of chinook salmon released into Bingham Creek, a total of 10,020 were recovered at the weir. A total of 946 (9.4%) smolts had predator mark(s). Of the marked fish, 923 (97.6%) were classified as rake marks, and 23 (2.4%) were blunt v-shaped marks presumed to have been caused by birds (probably kingfishers).

Data from the two releases were analyzed separately, as the proportion of marked fish was greater in the first release group (42.6%) than the second (24.9%). This suggested different levels of predation (Schroder et al. unpublished data). In the first group the proportion of marked fish recovered from each rearing vessel was not correlated with survival to weir ($R^2 = 0.02$, $F_{1, 12} = 0.308$, $P > 0.50$; Fig. 1). In the second group there was a significant positive relationship ($R^2 = 0.63$, $F_{1, 12} = 21.28$, $P < 0.01$; Fig. 1). Thus, fish from tanks with greater total survival were more likely to have escaped from predators than fish from tanks which produced fewer survivors.

Vulnerability to Cutthroat Trout

There were no significant interactions ($P > 0.05$) among the main effects of rearing treatment, structure, or trial round on the number of juveniles surviving predation by cutthroat trout. Significant differences existed among the seven treatments in the number of smolts surviving predation ($F_{6,18} = 2.86$, $P = 0.012$). A post-hoc comparison of the treatments revealed that survival of fish grown in the limited-complex environments (84.2%) was significantly higher than that (59.6%) of fish raised in tanks with a combination of cover and in-water structure ($P = 0.003$). There were no other significant differences among rearing treatments for any other paired comparisons (Fig. 2). The total number of smolts from all treatments surviving predation differed among the four trial rounds ($F_{3,112} = 7.81$, $P < 0.001$). Fewer smolts (59.7%) survived round 1 than survived round 2 (78.0%, $P = 0.015$) or round 4 (82.1%, $P < 0.001$). The presence of structure (i.e., two denuded conifers in the bioassay enclosures) had no effect on the number of smolts eaten by cutthroat trout ($P = 0.485$).

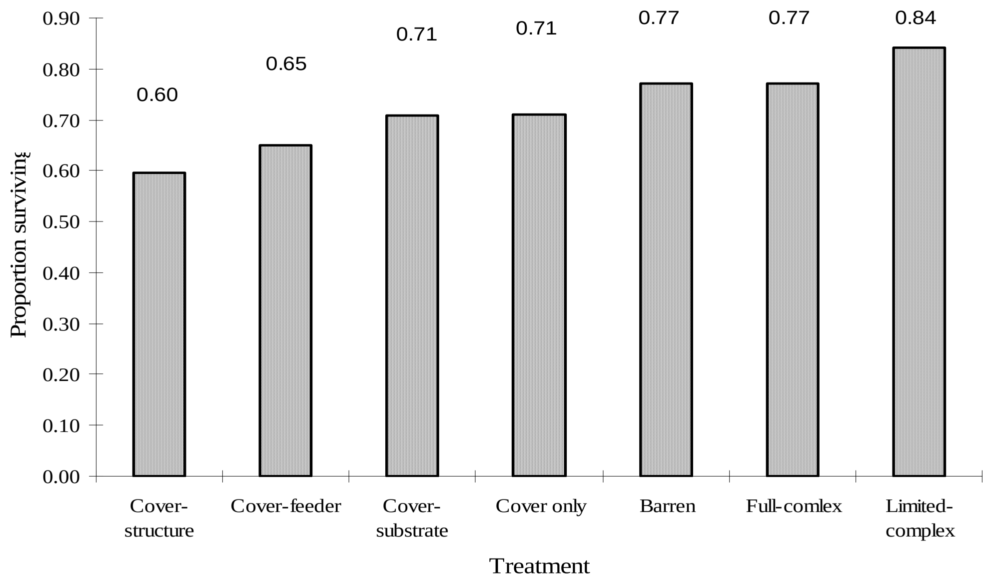

Figure 1. Proportion of smolts surviving 4 days exposure to cutthroat trout predators in sections of a gravel-bottomed raceway. Bars represent mean survival (+ 2 s.e.) of 24 trials.

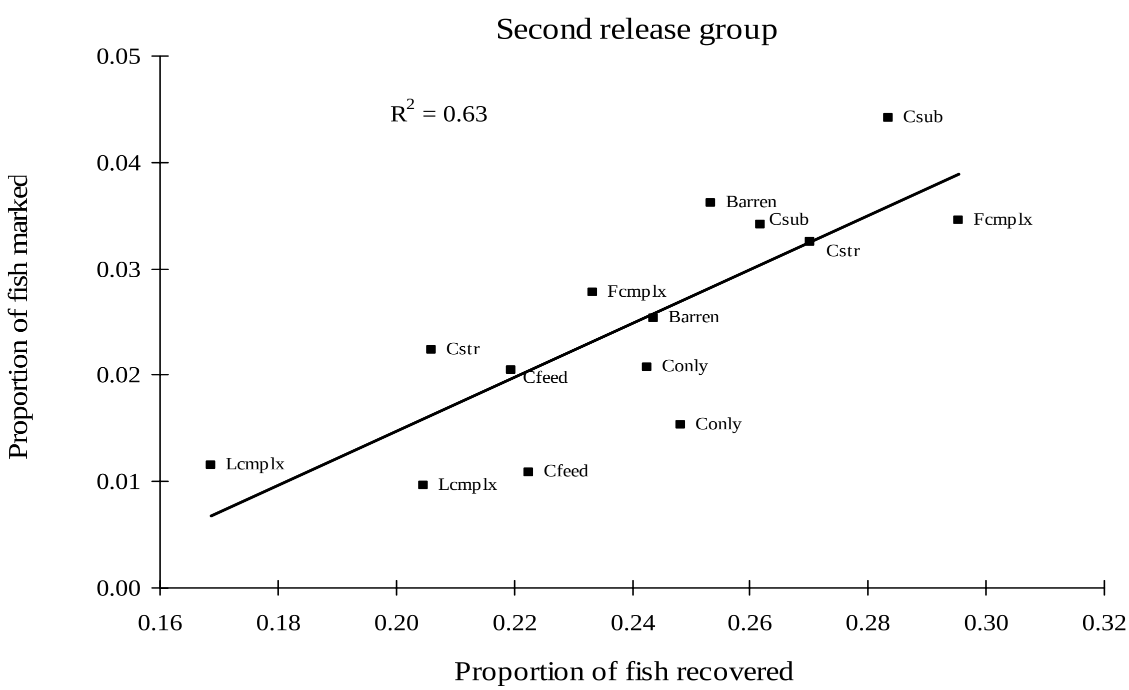

Figure 2. Proportion of smolts recovered with predator-induced marks (Y-axis) against the proportion recovered from individual rearing tanks (X-axis). Rearing-tank treatments are shown with corresponding data points.
Key: Fcmplx - full-complex; Lcmplx - limited-complex; Conly - cover only; Cstr - cover-structure; Csub - cover-substrate; Cfeed - cover-feeder; and barren.

Vulnerability to Avian Predators

A total of 20 trials (4 in each of the 5 cages) was attempted. In five of the trials nearly all (> 95%) of the fish were eaten before the cages could be covered, and in two trials few (< 5%) were eaten after 5 days. Thus relative vulnerability could be assessed in only 13 trials.

The number of fish eaten in each of these 13 trials varied widely (14-89%). This reduced the statistical power to detect differences in relative vulnerability among treatments. In fact there were no significant survival differences between treatments ($P > 0.05$). It appears that once the kingfishers began to prey on the smolts, they were effective in capturing all or nearly all within 24 hours. Thus it is necessary to redefine the experimental protocols, with perhaps the addition of submerged covers, if this type of predation bioassay is to be successful.

Discussion

The data from experiments with predator exposure are the first to demonstrate that structural modifications to hatchery rearing vessels affect vulnerability of the fish to predators. Random tank effects (i.e., those not attributable to the rearing treatments) were not analyzed in the experiments with cutthroat trout because of the small number of fish (only two or three) taken from each tank in each experimental trial. This was the same, regardless of the facts that the placement of individual tanks in the rearing area was randomized, all tanks were cleaned on the same day each week and within four hours of one another, and food ration, feeding times, and number of feedings were similar for all tanks. Thus, it is concluded that the results reflect differential effects of experimental rearing environments on the vulnerability of smolts to cutthroat trout.

In post-hoc paired comparisons, significant differences in vulnerability existed between the cover-structure (59.6% survival) and the limited-complex (84.2% survival) treatments (Fig. 2). Fish grown in the limited-complex treatment, with survivals similar to those of the full-complex treatment (77.0% survival), may have developed cryptic coloration patterns which reduced their chances of detection by the predators. The dark mesh substrate, pea gravel, and submerged camouflage cover probably combined to produce this effect. Fish grown in the barren treatment had the same survival (77%) as those grown in the full-complex treatment, and may also have benefited from improved coloration. In explanation, the absence of overhead cover on the barren tanks allowed substantial, multi-colored (primarily brown and green) algal growth on the side walls and bottoms of these tanks, and this did not occur in any of the treatments with overhead cover. The poorest surviving group, cover-structure, had very limited algal growth and did not contain a dark, multi-colored substrate.

Based on previous studies (White 1936, Donnelly and Whoriskey 1993, Matity et al. 1994) the incidence of predator marks on fish recovered after a 21-km migration in Bingham Creek could be used as an index of predation pressure. This would help to clarify the role of vulnerability in differences in postrelease survival. However, the assumption that predator marks reflect predation pressure on the population did not hold for this study. In the second release group there was a significant positive relationship between the proportion of fish from a given rearing vessel that survived downstream migration, and the proportion of those that sustained predator marks. This suggests the ability of these smolts to survive their downstream migration depends upon their ability to escape the grasp of a predator once captured, and the incidence of predator marks does not reflect predation pressure on the population - which would have been obviated by a negative relationship. The poorer overall survival of smolts from the second release group (28%) compared with the first (46%) suggests predation pressure was greater, which may account for the significant relationship between survival and mark rate in the second release and not the first.

Predation marks have been used as an indicator of predation risk in field studies of fathead minnows (*Pimephales promelas*: Matity et al. 1994), and Atlantic Salmon (*Salmo salar*: Donnelly and Whoriskey 1993). Matity et al. (1994) reported that breeding male minnows had a higher mark rate than non-breeding males or females, and these findings coincided with anti-predator behavioral responses. Donnelly and Whoriskey (1993) inferred that differential predator mark rates reflected differential predation risk in hatchery and wild Atlantic salmon. Neither of these studies, however, empirically tested the assumption that frequency of predator marks was inversely related to predator avoidance ability. The study data suggest that such assumptions must be tested for the predator and prey populations under investigation before conclusions regarding prey vulnerability using this surrogate measure of predation can be drawn.

Acknowledgements

The authors thank Joel Jaquez and Robert Redi for their cooperation at the Bingham Creek Hatchery; Lang Nguyen for culturing the chinook salmon, and marking and decoding the otoliths; and Jeff Grim and Dana Anderson for decoding the otoliths. This study was funded by the Bonneville Power Administration.

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Section 5

DEVELOPMENT OF A RACEWAY EXERCISE SYSTEM FOR FALL CHINOOK SALMON

by

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Introduction

Fish culturists may be able to increase salmon postrelease survival by exercising them in high velocity currents prior to release. Salmonids are typically reared in raceways with velocities of 1 cm/s, or less, designed to settle out pathogen-containing feces and debris (Pennel and McLean 1996). Research has demonstrated that the exercise provided by higher velocity currents (> 5 cm/s) has several benefits for cultured fish. Exercised salmonids both grow faster and have better food conversion than conventionally-reared fish (Christiansen et al. 1989, Christiansen and Jobling 1990, Christiansen et al. 1992). Regular exercise also improves swimming performance (Besner and Smith 1983, Leon 1986, Schurov et al. 1986a) and should enhance the ability of fish to escape predators. Most importantly, the postrelease survival of exercised fish has generally (Burrows 1969, Wendt and Saunders 1972, Cresswell and Williams 1983, Leon 1986, and Schurov et al. 1986b), but not always (Lagasse et al. 1980, Evenson and Ewing 1993), been higher than that of unexercised fish. These benefits are usually generated by exercise programs which force salmonids to swim at velocities greater than one body length/second for 1 hour a day for at least a 2-week period.

The preferred approach would be to exercise salmonids in high velocity single-pass raceways which flush pathogens and debris out of the vessel. However, for smolt-size fall chinook salmon, the generation of one body length/second (about 8 cm/s) exercise velocities in single-pass raceways usually produces unacceptable fish culture and water use conditions. For example, lowering water depth in a standard raceway to produce exercise velocities of one body length/second will increase rearing density by a factor of five or more. Alternatively, increasing water flow to a standard raceway by the five or more times required to generate exercise velocities produces plumbing, pumping, and water consumption costs most existing facilities cannot support. Finally, even the combination of these approaches may fail to achieve the one body length/second exercise velocity required by yearling salmonids.

The 16-cm/s exercise velocity required by a 30-g (15-cm fork length) yearling salmonid can be achieved in multi-pass systems (e.g., circular tanks and Burrows ponds). However, the replacement of existing standard raceways with these types of vessels would be economically unacceptable. Therefore, a recirculating design, similar to the Burrows pond, was developed that can be retrofitted to existing raceways. This report describes both: 1) design and operational parameters of this exercise system retrofitted to a rectangular tank, and 2) some preliminary effects of the system on fall chinook salmon.

Methods

The prototype recirculating design was installed and tested in six rectangular troughs at the Manchester Research Station freshwater salmon culture facility. The design uses the energy from inflowing water and low-energy pumps to circulate water around a central partition. Semi-elliptical screens are fitted to each end of the raceway to reduce the resistance of angled corners. Both the reduced cross-sectional area and the principle of inertia enable this design to produce the swift water velocities needed to exercise yearling chinook salmon. Each trough (5.5 × 0.6 × 0.6 m deep) was operated with a 0.3-m water depth (Fig. 1). The center of each trough was divided by a long aluminum divider (4.9 m) which ran parallel to the trough sides. There was an open space of 29.2 cm between the divider and each end of the trough. Semi-elliptical drain screens fabricated from perforated plate were fitted to each end of the trough to smooth out water flow. A dual inlet system delivered water to the center of the trough parallel to each side of the divider, generating a clockwise flow pattern within the trough. Two powerhead pumps (75 W) were installed in the tank to boost the recirculating flow around the divider. Water drained from the trough via holes (5.1 cm in diameter) located in each of the four bottom corners.

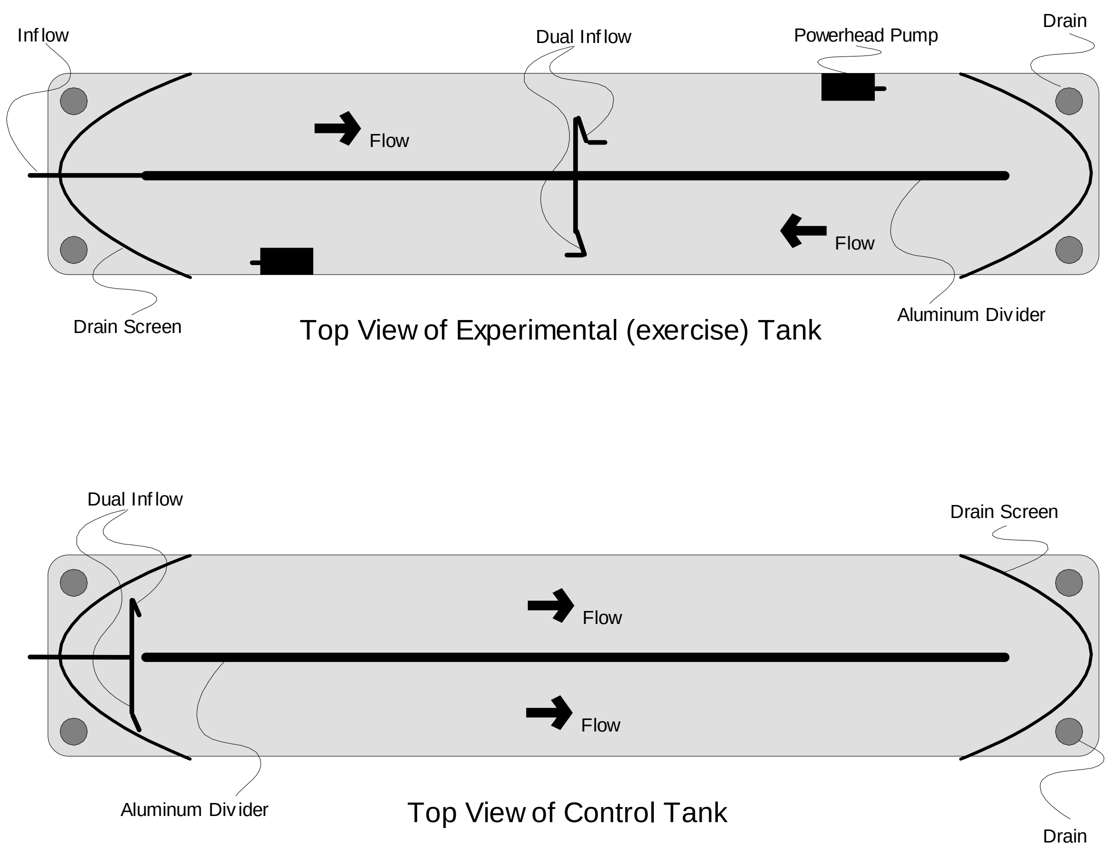

Figure 1. Top view of control and exercise troughs.

In summer 1998, a preliminary experiment on the effects of exercise on juvenile fall chinook salmon was initiated. The research was conducted in 12 rectangular troughs, half of which were designated as control troughs and the other half as exercise treatment troughs. The study was conducted with fall chinook salmon obtained from the WDFW Minter Creek Hatchery as swim-up fry that were reared in the NMFS Beaver Creek freshwater raceways until the fish were placed in the study. Seven hundred and fifty underyearling fall chinook salmon were ponded into each of the 12 raceways on 1 July 1998. A single pump was installed and turned on in each raceway on 13 July 1998. On 23 July 1998 the second pump was turned on. On 5 August 1998 a current velocity meter was used to record current velocity three times at six locations in the raceways with both pumps on.

Each trough was supplied with raw Beaver Creek surface water (at least 40-L/min) which normally carries a variety of salmonid parasites and pathogens. The treatment group was exercised 24 hours a day for a 14-day period in water velocities of 18.0 to 38.3 cm/s. The controls were never exposed to water velocities greater than 1 cm/s, except during the occasional brief draw-downs which occurred when the troughs were cleaned. In mid July, a pathogen outbreak (pathologists did not diagnose cause) resulted in increased mortality in the exercise troughs. On 27 July 1998, the exercise regimen was cut back to only 2 h/d. This new protocol was continued for an additional 60 days, until postrelease survival could be evaluated. Except for the exercise regime, the handling and husbandry of fish in both treatments was identical and followed standard salmon culture protocols.

Although there was concern that the pathogen outbreak might confound the results, it was decided to go forward with the postrelease survival evaluation. The effect of the rearing treatments on postrelease survival was compared by releasing fish from both treatments into Olalla Creek (a local Kitsap County stream). Fish were recaptured at a temporary fish collection weir (47° 25' 35" N and 122° 34' 19" W). For these evaluations, a representative sample of 80 fish from each trough was anesthetized in tricaine methanesulfonate (MS-222) and marked with a visible green microsphere photonic tag injected with the aid of compressed CO_2 and a panjet inoculation gun into one of the pelvic fins. Control fish were tagged on the left fin, while exercised fish were tagged on the right fin. Prior to release, all fish were checked to ensure the tag was retained. After being allowed time to recover from the effects of tagging, an equal number of fish from both treatments were combined into a common hauling container and trucked to a release site at least 0.5 km above the weir. Two releases (9 and 11 September 1998) were made into Olalla Creek (47° 27' 4" N and 122° 34' 44" W) for a total of 413 tagged test and control fish released (826 total fish released). The weir was checked daily and the number of fish with left or right pelvic marks recorded. The recaptured fish were then released downstream from the weir. On 29 and 30 September 1998, several sections of Olalla Creek were electrofished. The weir was kept in operation through 30 September 1998. The postrelease survival of fish from the two treatments was compared with a 2 × 2 contingency table analysis.

Results

The current velocities in the control troughs were less than 1 cm/s. Without fish present the current velocities in the experimental troughs ranged from 9.1 cm/s to 24.4 cm/s. The addition of fish to the experimental trough nearly doubled the current velocities, producing flows which ranged from 18.0 to 38.3 cm/s (Fig. 2).

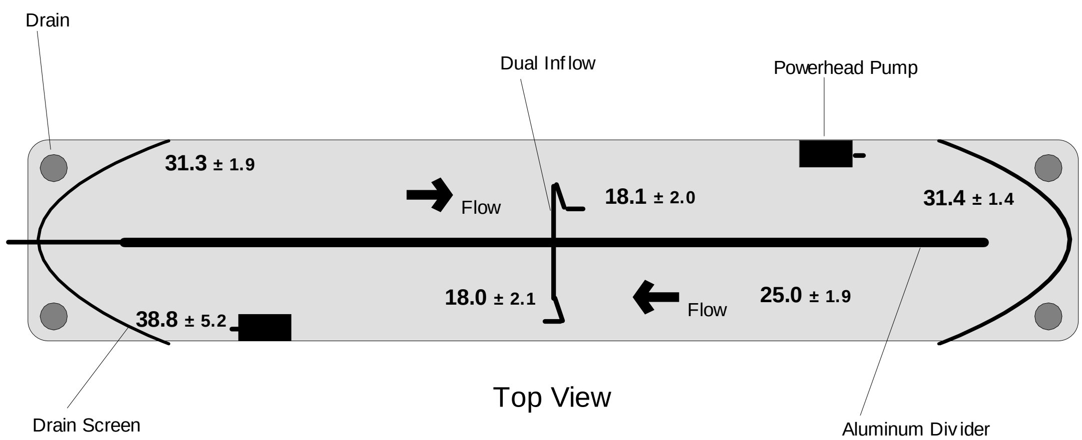

Figure 2. Mean current velocities (cm/s, n = 3) with standard deviations, as measured in exercise troughs with fish present.

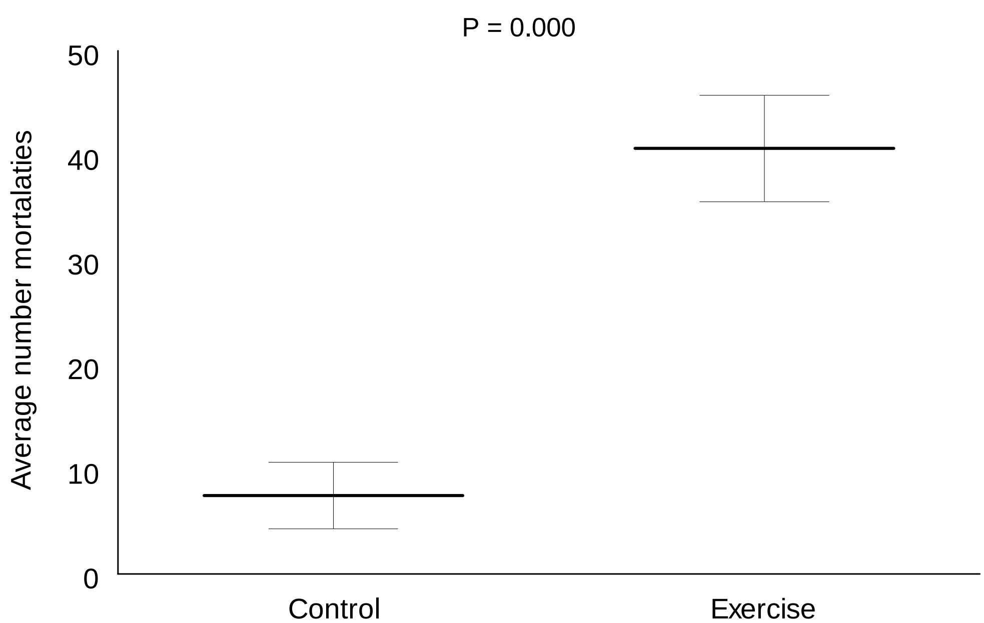

Figure 3. Average in-culture mortality (mean with standard error bars) in control (n = 6) and exercise (n = 6) troughs through 31 August 1998. Probability values (P) are based on *t*-tests.

The surface water supply to the rearing facility was warm (12.6 - 18.6° C) during the study period. The pathogen outbreak that occurred produced mortality that was statistically significant ($P < 0.001$) and four times higher in the exercise than non-exercise troughs (Fig. 3). This higher mortality rate within the exercise troughs continued during the late July and early August period when the exercise program was temporarily suspended and the fish were fed teramycin feed and given a formalin bath.

Control and exercised fish grew at similar rates. When the fish were tagged, exercised fish were slightly, but not statistically significantly ($P = 0.242$), shorter than control fish (Fig. 4).

The postrelease recovery of both exercised (15.3%) and non-exercised (16.2%) fish was similar and very low (Fig. 5). There was no significant ($P = 0.702$) survival difference detected between the two rearing treatments.

Discussion

The exercise system successfully generated current velocities that could be used to exercise chinook salmon. Even the low-end velocity of 9.1 cm/s was sufficient to exercise a typical 5-g fall chinook salmon smolt at more than one body length per second. The 18.0 cm/s velocity was sufficient to exercise a 30-g spring chinook salmon smolt at more than one body length/s. In the study, two 75-W pumps were used to produce the exercise velocities in each 0.09 m^2 cross-sectional area raceway. This suggests that, to achieve similar velocities in a production raceway, it would require about 1,666 W/m^2 of cross-sectional area. Thus, a standard 8' × 80' (2.4 × 24.4-m) raceway with 0.6 m of water depth would require only 1,200 W of electrical energy to generate similar velocities (roughly equivalent to a small portable electric space heater). A standard 10' × 100' (3.1 × 30.5 m) raceway, with 0.9 m of water depth, would require only 2,250 W of electrical energy. The key to this energy efficiency is the use of inertia and the tendency of water to push-pull itself around the central barrier.

The increased mortality associated with fish reared in the exercise troughs is of major concern. It is not clear if the increased mortality was due to physical stress associated with exercise or the multi-pass nature of the flow in the experimental troughs. In either case, it was clear that, that under the study conditions, exercising salmonids in the system was detrimental to their health. If there was a beneficial effect of exercise it was masked by the pathological problems experienced by the exercised fish. The study is currently being repeated with a less extreme exercise regime which, hopefully, will produce different results.

In summary, it is possible to achieve exercise velocities in raceways which are economic in terms of energy costs. However, from these preliminary results, it is not possible to draw any conclusions regarding the benefits of exercise in multi-pass systems. Future research should identify which exercise protocols are most useful for improving postrelease survival without the risk of losing fish to outbreaks of pathogens.

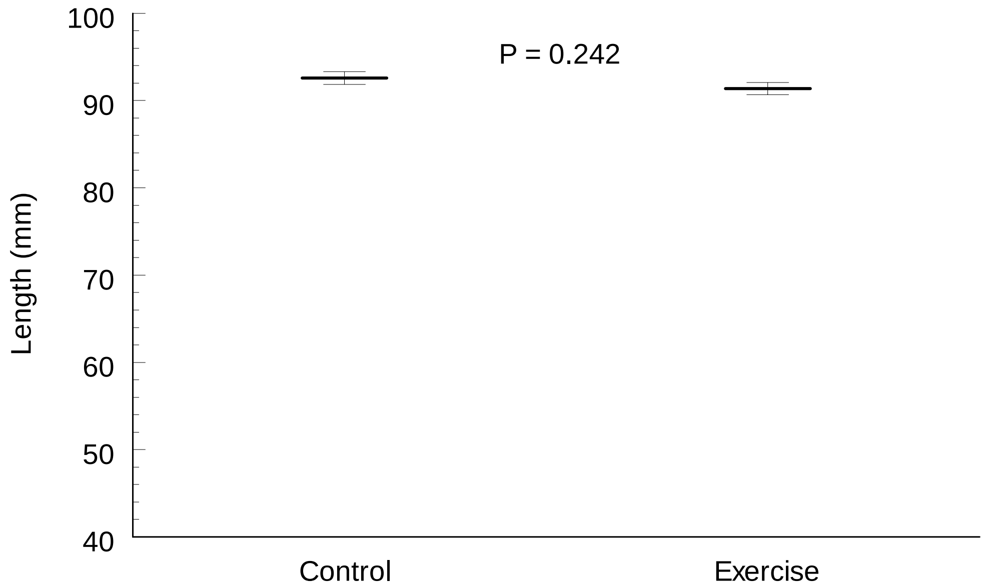

Figure 4. Mean fork length (with standard error bars) of control (n = 120) and exercised (n = 120) fall chinook salmon at tagging. Probability values (P) are based on *t*-tests.

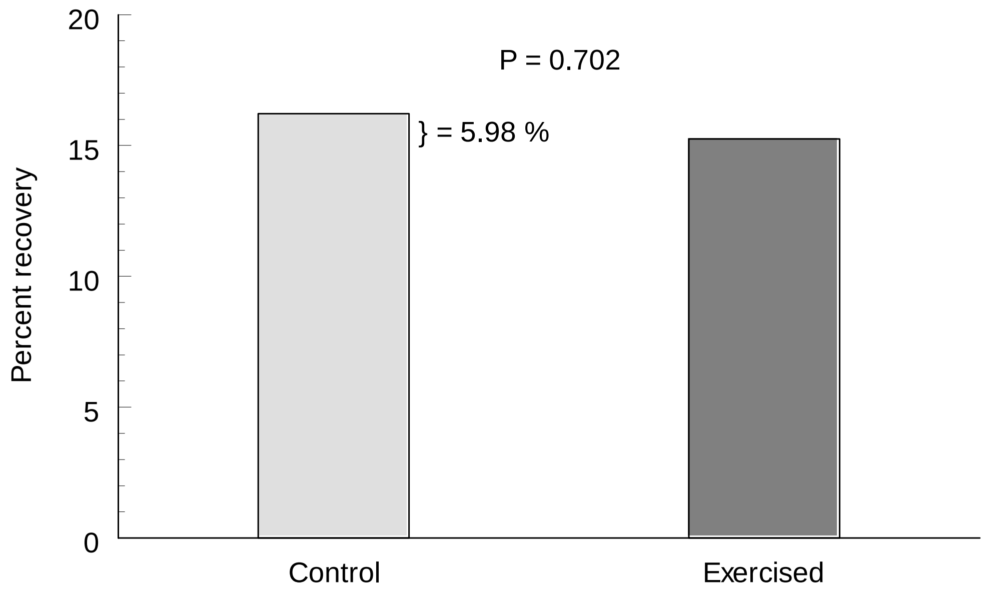

Figure 5. Percent recovery of control and exercised fish at Olalla Creek weir. Probability values (P) are based on chi-square analysis.

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Section 6

EFFECTS OF PREDATOR AVOIDANCE TRAINING ON THE POSTRELEASE SURVIVAL OF FALL CHINOOK SALMON

by

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Introduction

Predator avoidance training may be useful for improving the postrelease survival of hatchery-reared salmonids (Maynard et al. 1995). Laboratory studies have indicated salmon rapidly learn to recognize and avoid predators after observing attacks on conspecifics (Patten 1977, Thompson 1966, Olla and Davis 1989). Such experiences could increase their chances of survival during subsequent encounters with predators.

Thompson (1966) and Kanayama (1968) demonstrated that survival of young chinook and chum salmon in natural and artificial streams was increased by conditioning them to avoid models of predacious rainbow trout. This study attempts to demonstrate that postrelease survival of young fall chinook salmon is increased by conditioning them to avoid live predators.

Methods

Ninety-six thousand swim-up fry of fall chinook salmon, donated by the WDFW Minter Creek Hatchery, were transported to the NMFS Manchester Research Station. They were then systematically divided into six equal lots and placed in one of six outdoor pilot-scale raceways ($6.4 \times 1.5 \times 1$ m, with 0.6-m water depth) in the Station's freshwater facility. Fish in three raceways received experimental predator avoidance training. Fish in the three other raceways acted as controls and were tightly covered with nets to ensure no outside interference by predators. Except for the predator avoidance training, fish in both treatments received identical husbandry and standard rearing protocols for salmon.

The predator avoidance training employed a diverse array of predators to ensure the fish were exposed to at least one species they would encounter after release. It was also an opportunity to compare the suitability of each predator for conditioning avoidance behavior in hatchery-reared salmon.

In March 1997, predator training was initiated by uncovering the three experimental raceways to allow local fish-eating birds access to the fish. Although a young great blue heron occasionally fished the raceways, it disappeared within a few weeks and was not observed again. Belted kingfishers occasionally flew overhead during the study, but were never observed to fish in the raceways. The potential for any in situ training exposure to natural predators was therefore deemed impractical.

More routine predator training sessions began in April by placing caged hooded mergansers, largemouth bass, and brown catfish in the raceways. The cages ($1.6 \times 1.1 \times 1.1$ m) were frame constructed with PVC-pipe (1" diameter) and fittings, and covered with net. The mesh size (3.8 cm^2) allowed fry to swim freely in and out of the cage, while confining the predators. The top half of each cage was out of the water so that the birds would not drown. Empty cages were also placed in the raceways to train the fish to associate predation events with predators, and not the cage itself.

Two phases of cage training were carried out. In the first phase, a pair of hooded mergansers was used for seven training periods of 50 minutes duration in late April. In nearly every session, the mergansers were removed before they ceased fishing. This ensured the fry experienced nearly continuous negative reinforcement from these predators. In the second series, two largemouth bass and one brown catfish were used for a week. Before being used, the appetite of each predator for chinook salmon fry was demonstrated by their consuming fry in pre-training evaluations. Both phases were completed by mid-May.

The effects of predator avoidance training on postrelease survival were evaluated in Curley Creek, a tributary of Puget Sound. The releases were made with representative samples of fish from each of the six raceways. The samples were removed from the raceways, transferred to six circular tanks (1.5-m diameter), and held until they were released in July. About three weeks before the first release, each fish was measured for fork length (to the nearest 1 mm), weight (to the nearest 0.1 g), and tagged with a passive integrated transponder (PIT tag). On 25 and 30 June one unconstrained largemouth bass was placed in each of the tanks containing the conditioned fish and allowed to prey for eight hours.

Releases began on 3 July 1997. A total of 51 fish were transported and released into each of two tributaries of Curley Creek. Each release site was about 1.3 Km upstream of the Curley Creek smolt collection weir. The release program attempted to minimize any effects of contagious behavior by releasing fish from only one rearing treatment in each tributary on a given release day. Contagious behavior is a form of social learning, where naive animals mimic the behavioral displays of their more experienced peers to predators, food sources, and new stimuli. The possibility of fish from different rearing treatments meeting each other at the release sites was further reduced by making releases every 48 hours. Any effects of the tributary choice were further reduced by alternating each tributary from one release to the next. A total of 511 control and 510 predator-trained fish were released in 10 equal releases. The difference in recovery between the two treatments was compared with contingency table analysis.

Results

The fry rapidly learned to avoid mergansers in the predator conditioning trials. Before the introduction of the birds into the cage in a raceway, the fry readily swam in and out of the cage. However, after three training sessions with mergansers few fry continued to enter the cage. By the fifth session almost no fry entered the cage at all, and nearly all the fish remained at least 15 cm from the cage. Initially, mergansers consumed an average of more than nine prey per training session. This declined rapidly to less than six prey per training session as the fry became conditioned to avoid the birds.

The predator avoidance behavior of the fry to largemouth bass and brown catfish differed from that for mergansers. Few fry entered the cage when bass and catfish were first introduced, but they began to enter within a day. After a week living with these piscivores there were as many fry residing in the cage as out. This difference in distribution over time may be related to the different hunting tactics. The mergansers continuously pursued their prey. The bass and catfish, on the other hand, passed their time either holding in place or slowly cruising around the cage perimeter. Furthermore, although proven predators, their appetites seemed not as great as the mergansers. The largemouth bass used in the final conditioning before release, for example, consumed on average only five fish during the overnight training period.

Predator avoidance training did not appear to affect fish growth. The average fork lengths of fish in the two treatments (Fig. 1), measured when the fish were tagged, did not significantly (P = 0.702) differ. Neither did the weights (Fig. 2) of fish (P = 0.110).

In this experiment, predator avoidance training increased postrelease survival. The post-release recovery of predator-conditioned fish was significantly higher (P = 0.046) than that of control fish (Fig. 3). The relative survival [(% recovery experimental treatment - % recovery control treatment) / (% recovery control treatment)][100%] of predator conditioned fish was 26% higher than that of control fish. Within a week of the last release, the recovery rate of fish from both treatments had drastically dropped. Although the weir was operated into September, only 18.6% of all the fish released were recovered.

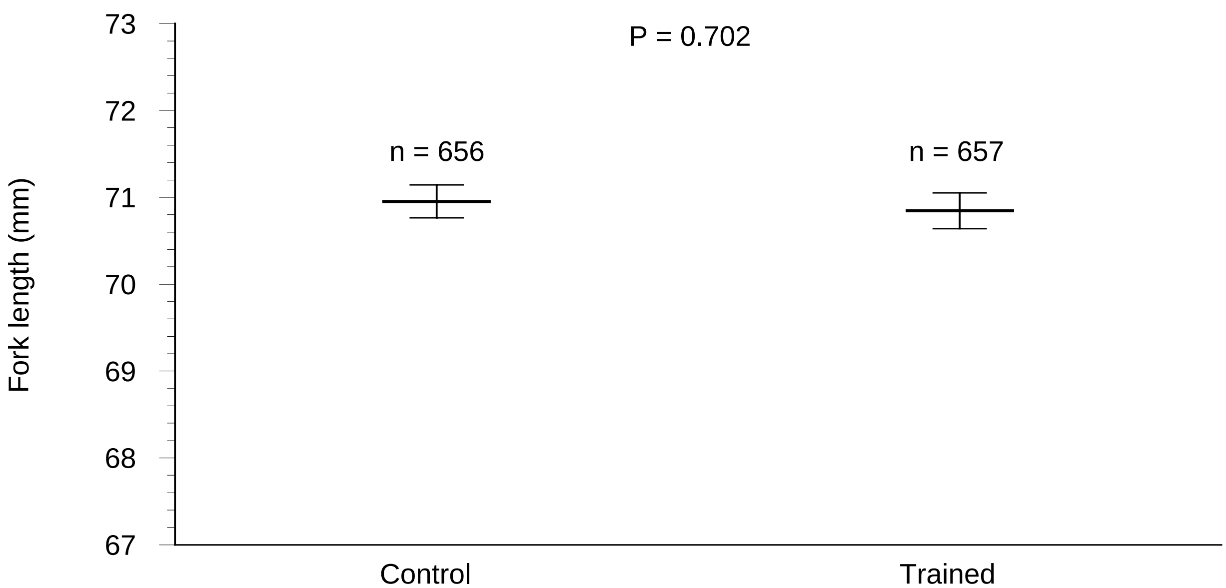

Figure 1. Mean fork length (with standard error bars) of control and predator trained fall chinook salmon at tagging. Probability values (P) are based on *t*-tests.

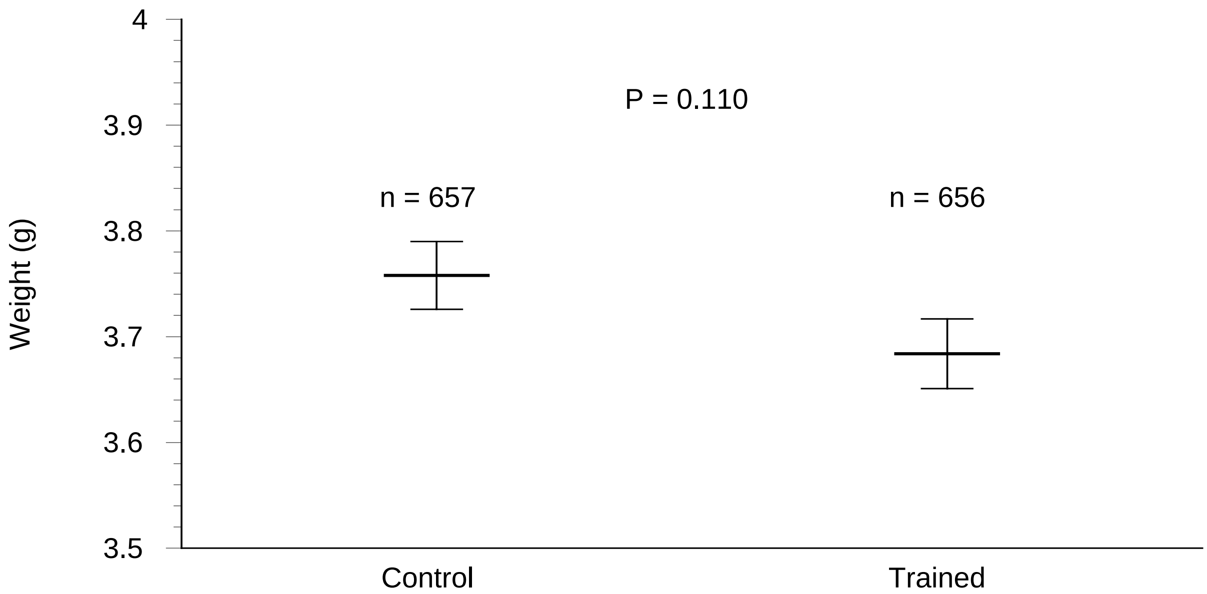

Figure 2. Mean weight (with standard error bars) of control and predator trained fall chinook salmon at tagging. Probability values (P) are based on *t*-tests.

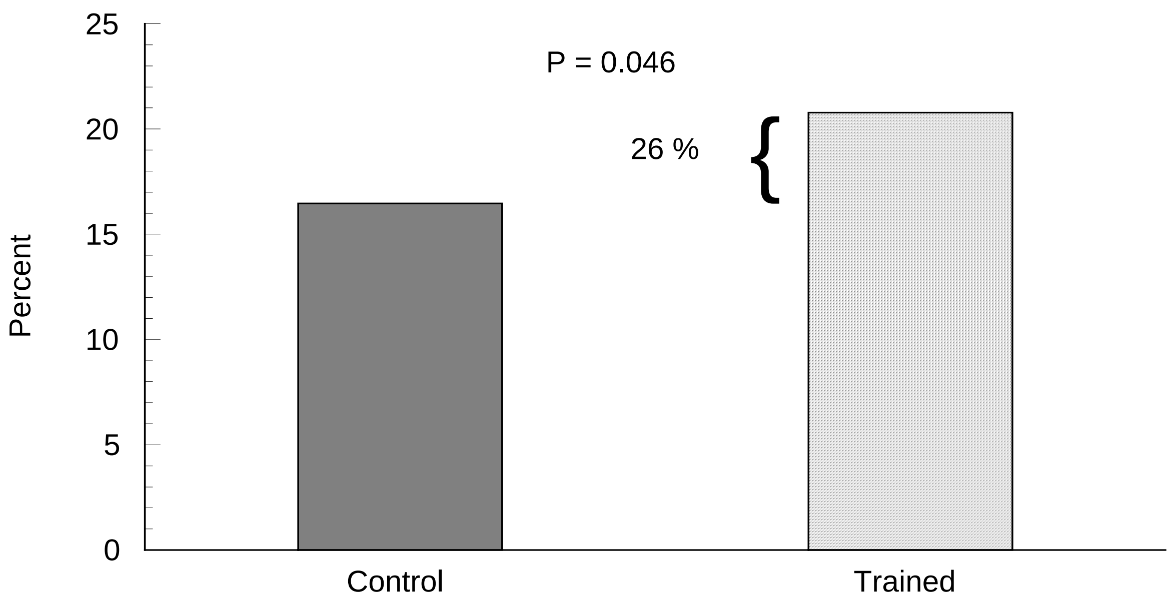

Figure 3. Percent postrelease recovery of control and predator trained fall chinook salmon at the Curley Creek weir. Probability values (P) are based on chi-square analysis.

Discussion

The study indicated that predator avoidance training with live predators can increase the postrelease survival of hatchery-reared salmonids. The benefits of training can be considerable, with postrelease survival of predator-trained fish in the current study being about 26% higher than untrained fish.

The predator avoidance training protocol used in this study required only a slight increase in operational costs. During training less than 2 hours of personnel time were expended per day in handling mergansers. The birds rapidly learned to enter the training cage, which was easily transported to and from the raceways. Once the cage was placed in the raceway it did not interfere with routine fish culture operations. A pair of hooded mergansers can be purchased for about $125, and it took less than 10 minutes of attention each day to maintain them in captivity. The bass and catfish have similarly low acquisition, handling, and maintenance costs. Therefore, the increased survival of predator-trained fish far outweighed the small increase in operational costs.

Although predator avoidance training is a useful tool for increasing postrelease survival, it need only be used at facilities which produce predator-naive fish. Hatcheries which allow predators to enter their ponds, with no bird nets or electric fences to provide protection, are probably already providing uncontrolled predation training.

Programs using production hatcheries to enhance a fishery or mitigate for habitat loss could derive several benefits by adopting predator avoidance training protocols. The most obvious is simply to use increased postrelease survival following training to increase the number of fish available for harvest. Secondly, the increased survival might also be used to reduce the number of fish which must be reared and released to produce an equivalent number of fish for harvest or to meet mitigation goals. Thirdly, greater survival could be used to lower operational costs (with fewer fish needing to be fed, marked, etc.) to produce an equivalent number of recruits to the fishery. Finally, the increased survival offered by antipredator training would permit facilities to meet their enhancement and mitigation goals while reducing the number of wild fish needed for broodstock and releasing fewer smolts to interact negatively with wild fish in the migratory corridor. These are two important considerations for enhancement practices in areas where they may impact endangered and threatened stocks.

The development of predator training protocols is in its infancy. Research is necessary to compare the conditioning stimuli of live predators with electrified models, similar to those used by Thompson (1966) and Kanayama (1968). Work is also required to identify which cues (visual, acoustic, chemical, or a combination) provide the information necessary for effective predator avoidance training. This research will not only refine techniques but will also provide non-lethal training protocols for the reintroduction of endangered and threatened stocks of salmon.

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Section 7

COORDINATING THE INTEGRATION OF NATURES VARIABLES INTO THE FORKS CREEK STUDY

by

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Introduction

New culture techniques must be developed for chinook salmon as less than 2% of these fish released from hatcheries survive to recruit to either the fishery or spawning population. Given that hatchery-produced salmon account for up to 80% of the fish in the fishery and that they are frequently the only source available for natural-run restoration, this is an unacceptably low survival rate. Fortunately there appears to be scope for improving hatchery-reared salmon postrelease survival, as their smolt-to-adult survival is much lower than that of wild-reared salmon. Salmonids produced with traditional fish culture techniques apparently lack many of the behavioral, physiological, and morphological characteristics needed to survive in the wild (see the review by Maynard et al. 1995). It may be possible to promote the expression of these wild characteristics by rearing salmon in a more natural hatchery environment.

Previous studies have been developing NATURES approaches consisting of seminatural raceway habitat, live food diets, exercise systems, predator avoidance training, underwater feed delivery systems, etc. (Maynard et al. 1995, 1996a, b, c, d). Seminatural raceway habitat composed of natural gravel substrates, artificial or natural vegetative structure, and overhead cover can be used to produce a more natural hatchery environment. In previous studies (Maynard et al. 1996c) and those described in this report the in-stream survival of chinook salmon reared in this NATURES raceway habitat was 25-50% higher than for fish reared in conventional raceways. It is unknown if this increase in in-stream survival leads to increased smolt-to-adult survival. Therefore in 1996, NMFS, Long Live the Kings (LLTK), WDFW, BPA, and the Weyerhaeuser Corporation initiated a multiyear production scale test to determine if rearing fall chinook salmon in seminatural raceway habitat also increased smolt-to-adult survival. The study was primarily funded by NMFS, WDFW, and LLTK. BPA participation was focused on coordinating information transfer of NATURES variables developed under BPA funding to the Forks Creek experiment. This section reports on the progress of the study through 1998.

Methods

The experimental facility was developed in 1996 with the installation of eight fiberglass raceways on a concrete pad at the WDFW Forks Creek Hatchery near Lebam, Washington. Forks Creek is a tributary of the Willapa River. The raceways (9.75 × 2.44 × 1.24 m high) maintained a water depth of 0.80 m. The interior of all eight raceways was originally dark gray (10 on the Kodak gray scale). In 1998, the interior of the control raceways was changed to a lighter shade of gray (2 on Kodak gray scale) to resemble the lighter color of concrete.

The control raceways were left unmodified for the most part. In all 3 years of operation, the top of each control raceway was covered with nine aluminum-frame panels (1.96 × 2.64 m) to prevent avian predation in the control tanks. The panels were fitted with white nylon netting (0.6 × 0.6 cm mesh) to prevent small predators, such as dippers (*Cinculus mexicanus*), from entering.

NATURES technology was transferred to the project so that the experimental raceways were fitted with resin rock paver substrate, a submerged Douglas fir tree structure, and covered with (U.S. military) camouflage netting. The resin rock pavers were tiles (0.6 × 0.6 m) fabricated with epoxy resin and river gravel (22 - 32 mm diameter). The river gravel was carefully selected to match the color of the sand and gravel found in Forks Creek. Structure was created by suspending five defoliated fir trees from a single horizontal cable running the length of each raceway, preventing them from touching the bottom. All needles were removed from each fir tree before weighting with rebar. The trees were suspended from the horizontal cable by vertical cables attached to each end of their trunk. Each free cable end was then attached to the horizontal cable with an interlocking spring snap so the trees could be easily moved or removed during cleaning. The raceways were covered with netting framed panels identical to the controls except a single layer of camouflage net was hung to provide 40% covered area along each side.

1997 Activities

In 1997, four experimental raceways were used for the study. On 24 February 1997, identical weights (approximately 54,000 fish) of fall chinook salmon swim-up fry were ponded into each raceway. The fish were reared following standard WDFW fish culture practices (Michak 1997). Each raceway was cleaned at least once every week with a commercial swimming-pool vacuum. All mortalities were counted and removed.

Every month a sample of 100 fish was removed from each raceway, weighed (to the nearest 0.001 g), measured (fork length to the nearest 1 mm), and means compared with *t*-tests. At least 30 fish in each sample were photographed with 400 ASA color slide film using a Nikon 8000S single lens reflex camera equipped with a micro lens (60 mm) and circular polarizing filter. The camera was mounted on a photographic light stand equipped with two quartz halogen lamps (300 W). The light was filtered through photographic gel to simulate daylight.

Before being photographed, the fish were anesthetized in MS-222 solution in black dishpans and then placed individually on a clear acrylic angled stand over a standardized blue background. The fish were photographed at least twice.

Each photograph was mounted in a standard plastic slide mount. This enabled it to be placed on a PVC plate (with the center drilled out) attached to the stage of a stereoscopic binocular. A fiber-optic light illuminated the slide from below. The image was then picked up by a Hyper HAD RGB color video camera. The video image was then captured and processed by image analysis software. For skin color analysis, a rectangular section of the caudal fin was examined on each fish for hue, intensity, and saturation values. These values were compared with *t*-tests.

Over a 2-week period in late April and early May 1997, approximately 51,000 fish in each raceway were coded-wire-tagged and adipose-fin-clipped for subsequent evaluation of survival (smolt-to-adult). The fish in each raceway received a unique batch code. These fish were reared on and then released from the hatchery in two paired releases on 7 and 9 June 1997.

It is anticipated from fall 1999 until 2003, the regional database of the Pacific States Marine Fisheries Commission (PSMFC) will be used to compare the recruitment to the fishery and relative survival to spawning of these conventionally- and seminaturally-reared fish.

On 4-6 June 1997, a subsample of 750 fish from each raceway was PIT tagged for an in-stream survival evaluation. The fish were returned to their raceway and allowed to recover from the effects of tagging. Later, they were transported in a white fiberglass tank to the upper watershed of Forks Creek (location 46° 30' 51" N and 123° 32' 8" W) where they were released in paired groups. Releases were made on 16 and 23 June 1997. Each paired group consisted of all the PIT tagged fish in one control and one seminatural habitat raceway. The fish were challenged to survive downstream migration to a weir located at the hatchery (46° 33' 26" N and 123° 35' 46" W), where they were recaptured and their PIT tag code interrogated. After all the codes were recorded, the fish were released below the weir to continue their migration to sea. Differences in the ratio in the number of fish recaptured to not recaptured were compared between the treatments with 2 × 2 contingency table analysis.

A subsample of 30 fish was removed from each raceway on 4-6 June 1997 for pathological analysis. These fish were euthanized in MS-222 and then dissected for evaluation. The condition of the spleen was observed and subjectively rated. The kidney was sampled. The posterior third of the kidney was removed and examined for the presence of *Renibacterium salmoninarum*, the causative agent of BKD, and the fluke *Nanophyetus salmincola*. Portions of the kidney were streaked on agar plates, incubated at 20° C, and examined after 24 hours for evidence of bacterial pathogens. *N. salmincola* cysts were analyzed with a *t*-test.

1998 Activities

In 1998, the number of raceways per treatment was increased from two to three. The inside walls and bottom of the control raceways were lined with a light gray fiberglass reinforced panel (2 on Kodak gray scale) to resemble the light gray color of concrete (1 to 2 on Kodak gray scale). The original resin rock pavers were replaced with new pavers made of resin that did not turn white when submerged in water. Except for these modifications the control and seminatural raceways remained the same as in 1997.

The second experiment was initiated on 27 January 1998. An equal weight of fall chinook swim-up fry (approximately 37,035 fish) was ponded into each of the experimental raceways. The fish were again reared following standard WDFW procedures. Beginning in February 1998, samples of 100 fish from each raceway were weighed and measured every month as before, and means analyzed with *t*-tests. The number of fish photographed was maintained at 60 per treatment but reduced to 20 per raceway.

Over a 2-week period in April 1998, at least 33,500 fish in each raceway were tagged and fin clipped for the evaluation of smolt-to-adult survival. These fish were then reared until 1 June 1998, when they were released from the hatchery below the Forks Creek weir. From fall 2000 to

2004, the PSMFC database will be consulted to compare the recruitment to the fishery and relative survival to spawning of fish from the two rearing treatments.

In spring 1998, the vertical position of the fish in each raceway was recorded using a grid and an underwater video system. The grid had four cells stacked vertically between the surface and bottom of the tank. Data were recorded on videotape (8 mm) for subsequent analyses.

On 1-2 June 1998, 30 fish from each raceway were sacrificed for pathological evaluation, which differed slightly from that carried out in 1997. The fish were first euthanized in MS-222. The fin condition was assessed, and fish with eroded or split fins were scored. The coelomic cavity was opened and the condition of major internal organs assessed. These results were compared with 2×2 contingency table analysis. The kidney was then sampled and evaluated as previously described for 1997.

On 1-2 June 1998, 500 fish from each raceway were PIT tagged for in-stream survival evaluations. These fish were transported and released in three paired releases at the same upper watershed location as in 1997. Paired releases were made on 10, 17, and 24 June 1998. Survival ratios were again compared with 2×2 contingency table analysis.

Results

A landslide in the Forks Creek watershed upstream of the hatchery intake occurred in early 1997. This resulted in heavy siltation during the first 6 weeks of rearing. Although the raceways were cleaned daily with a vacuum, thick sediment built up each day throughout this period. By late spring much of the sediment had been washed out, and the bottoms of the conventional raceways were clean of silt. There was no increased siltation load in 1998.

In 1997, the original pavers turned white after several days in water. This reaction appeared to be more physical than chemical. They also crumbled when taken out of the raceways. The resin rock pavers used in 1998 were a distinct improvement with the resin remaining transparent.

In 1997, it was necessary to remove the juvenile fish collection weir temporarily before PIT tag recoveries were complete due to heavy flooding. The weir was modified for the 1998 field trials and was not removed during the recovery period.

The resin rock pavers markedly reduced vacuuming time compared with that for cleaning loose gravel. Improvements in the vacuum heads and larger wheels to roll over the rocks enabled the seminatural raceways to be cleaned quickly. In general it took three passes with a seine net to catch almost every fish in seminatural raceways. It took two passes to net almost all the fish in conventional raceways.

The five fir trees suspended from a wire into each raceway were relatively easy to maintain and work around. The system enabled trees to be unclipped and rapidly removed when it was time to crowd the fish for sampling or removal. Although some branches were lost each season, the trees lasted for at least 2 years.

The camouflage net covers were also easy to work around. The covers were lifted and one side hung from a wire to provide easy access for feeding, vacuuming, or removing mortalities. For seining operations the covers were easily removed and temporarily stored next to the tank. The standing wall tanks and covers successfully eliminated all avian and mammalian predation from the study raceways, even though birds and otters were seen to prey on fish in the uncovered production raceways and ponds at Forks Creek Hatchery.

In 1997, although randomly distributed, the two fish groups differed in length and weight at ponding (Figs. 1 and 2). This difference in size had disappeared by the second sampling period. By the third sampling period, the conventionally-reared fish were significantly longer and heavier than the seminaturally-reared fish. Even after reducing the ration fed to conventionally-reared fish, they remained slightly larger than seminaturally-reared fish at the last sampling period (Figs 1 and 2).

In 1998, there was no significant difference in the length or weight of fish at either the first or second sampling periods (Figs. 3 and 4). By the third sampling period, the seminaturally-reared fish weighed significantly less, but were not shorter, than the conventionally-reared fish. In the fourth sampling period in April 1998, the seminaturally-reared fish were both significantly shorter and weighed less than their conventionally-reared counterparts. Although feed was withheld from the conventionally-reared fish to allow the seminaturally-reared fish an opportunity to catch up, conventionally-reared fish were still slightly larger than seminaturally-reared fish at the end of May 1998.

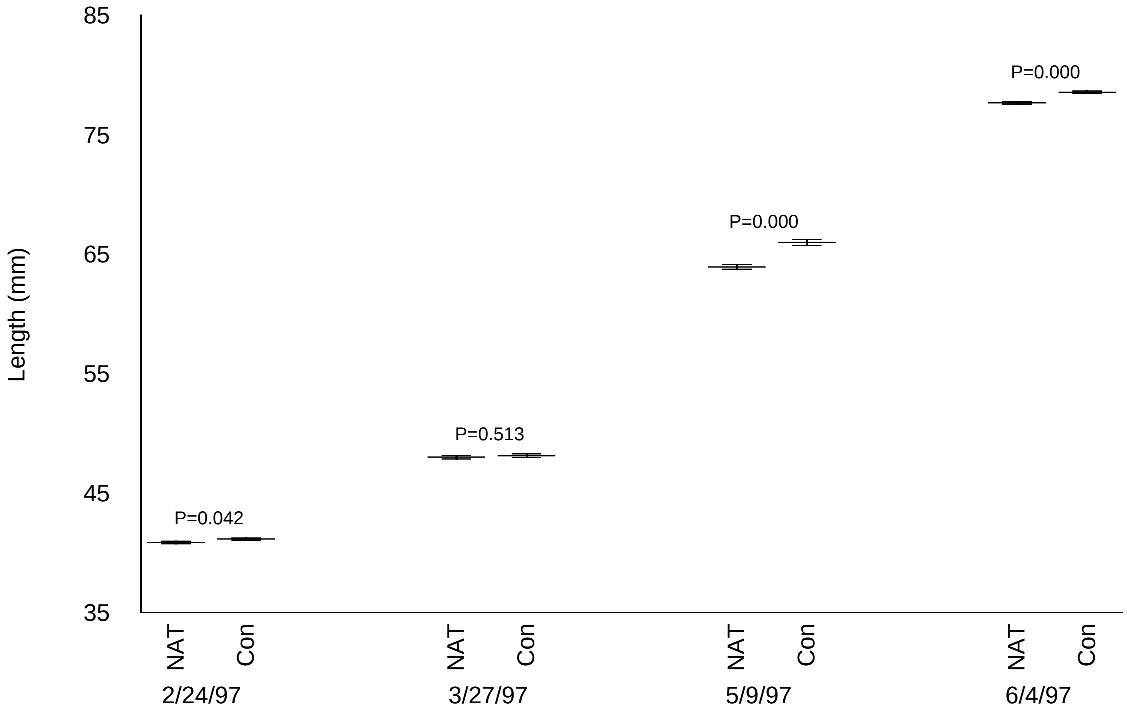

Figure 1. Mean lengths (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 200) or conventional (con, n = 200) raceways at Forks Creek Hatchery in 1997. P values are based on *t*-tests.

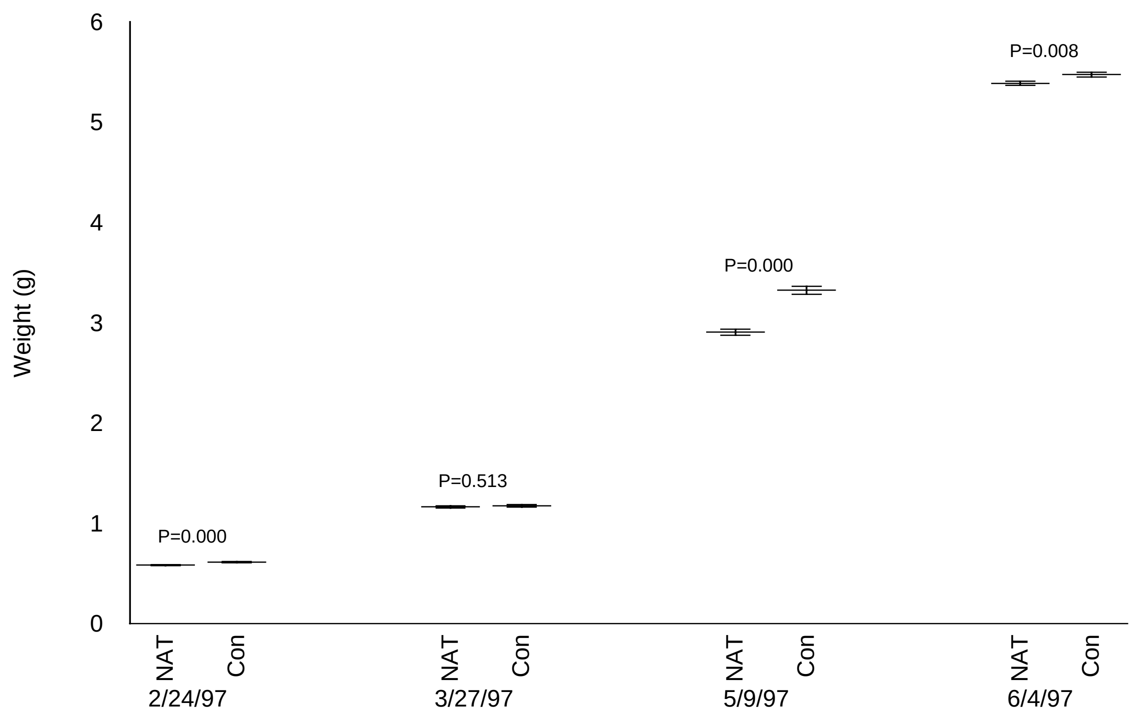

Figure 2. Mean weights (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 200) or conventional (con, n = 200) raceways at Forks Creek Hatchery in 1997. P values are based on *t*-tests.

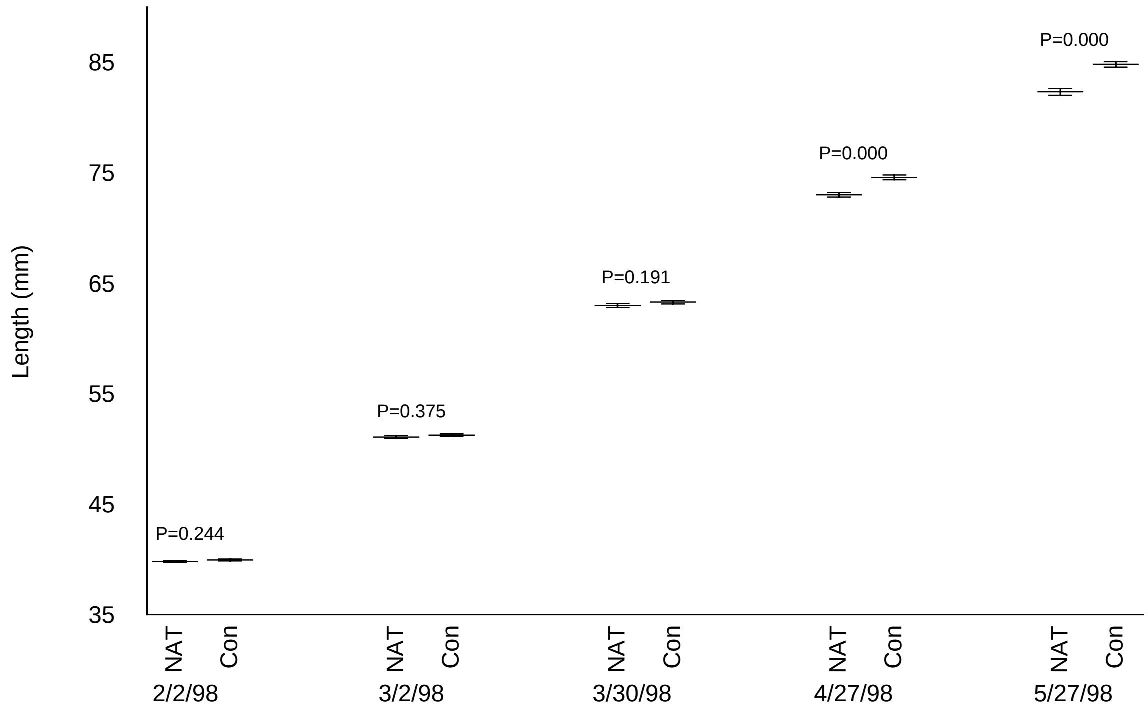

Figure 3. Mean lengths (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 300) or conventional (con, n = 300) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

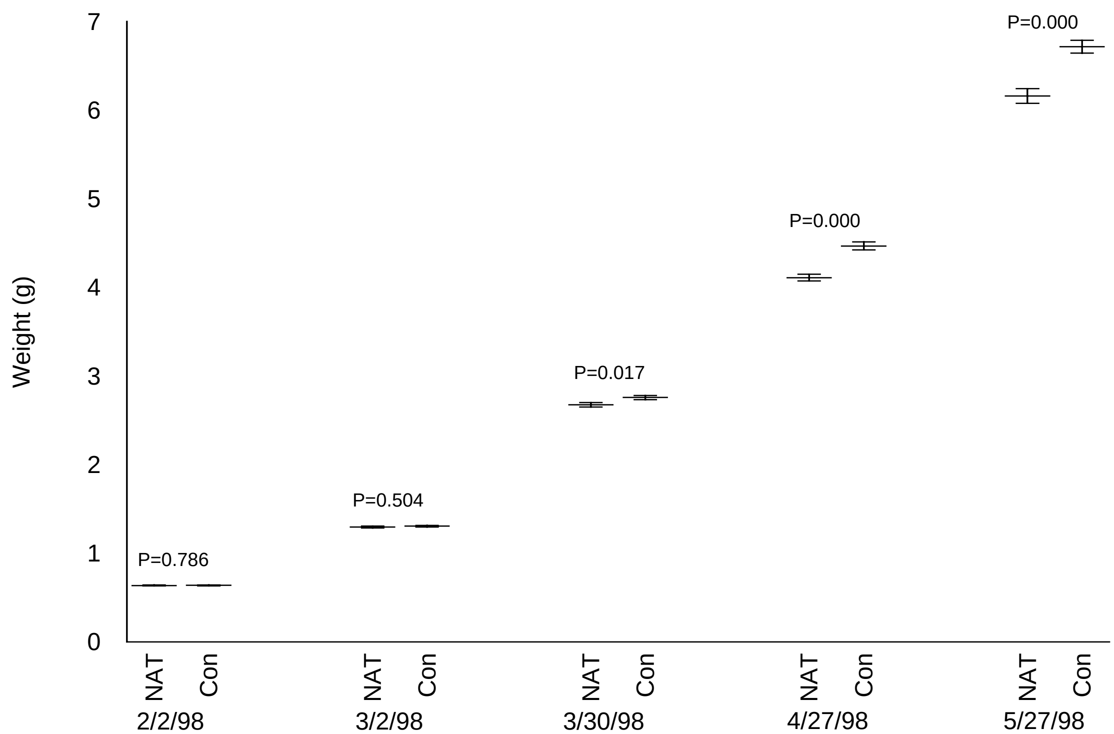

Figure 4. Mean weights (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 300) or conventional (con, n = 300) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

In 1997, fish were photographed the day they were ponded into the two rearing treatments. The skin color values for both treatments were similar at ponding (Figs. 5, 6, and 7)[2]. Statistically significant differences did not develop between the two treatments until the last sampling period in June 1997. Only two (hue and intensity) of the three color axes were significantly different from one another just prior to release. Subjectively, the seminaturally-reared fish appeared darker than the conventionally-reared fish. The caudal and pectoral fins of conventional fish were translucent, while the caudal fins of seminatural fish appeared brown. There also appeared to be much more chromatophore development in the ventral region of fish reared in seminatural rather than conventional raceways.

In 1998, photographs were taken when the fish had spent a week in their respective rearing treatments. This length of time in treatment was found to be insufficient for the fish to develop skin color differences that were statistically different (Figs. 8, 9, and 10). By March the skin color differences were statistically different, and these differences continued through the last sampling period in May 1998. Although all three color values were statistically different at intermediate sampling periods, only two of the three color axes (hue and intensity) were statistically different at the final sampling. In 1998, the subjective color differences between conventional and seminatural salmon appeared to be greater than in 1997. Again, seminaturally-reared fish had brown-tinged fins and more chromatophore development in the ventral region than conventionally-reared salmon.

The pathological data for the two rearing treatments were very similar in 1997. The kidney streaks from both rearing treatments produced no pathogen colonies on the TSA agar plates. Although *N. salmincola* cysts were observed in the kidney smears from both treatments, the average cyst counts were similar and not significantly different (Fig. 11). Enteric redmouth disease broke out in one of the control tanks in 1997, and produced some mortality. The fish in all four tanks were fed medicated (Romet) feed as soon as the outbreak was detected and diagnosed by WDFW fish health staff.

The fish were not checked for the protozoan *Ichthyobodo necator*, or given a formalin bath to prevent the high postrelease mortality this parasitic agent can cause when salmonids migrate into the marine environment.

In 1998 the health sampling program was extended to include gross assessment of fin condition and internal organs. In these observations, the percentages of fish with fin or kidney problems were similar for both rearing treatments (Figs. 12 and 13). However, abnormal spleens were observed in more conventional than seminatural fish (Fig. 14). As in 1997, none of the streaks on the TSA agar plates resulted in the growth of identifiable pathogen colonies. Enteric redmouth disease again broke out in one of the conventional raceways and all six raceways were fed medicated (Romet) feed. This immediately eliminated subsequent mortality problems

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[2] The reader should be cautioned to make comparisons only between treatment values for hue, saturation, and intensity (i.e., only look at relative differences between treatments). Values should not be compared between sampling dates, as sources for film and processing, and developing times were not always consistent from one sample to the next.

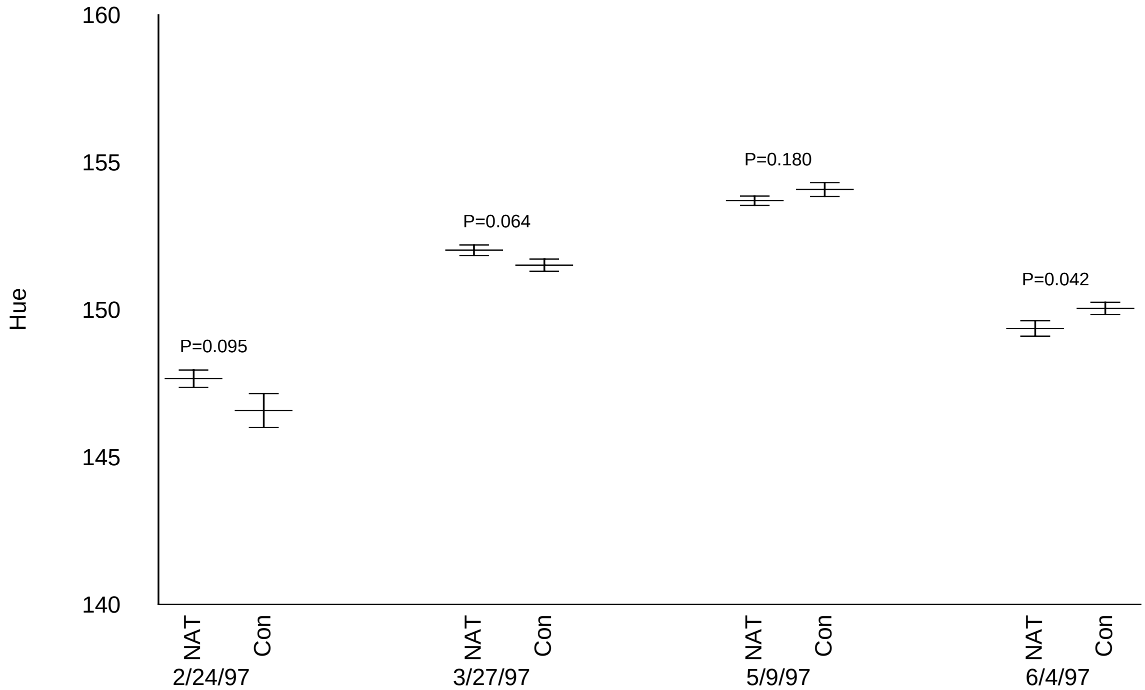

Figure 5. Mean hue values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1997. P values are based on *t*-tests.

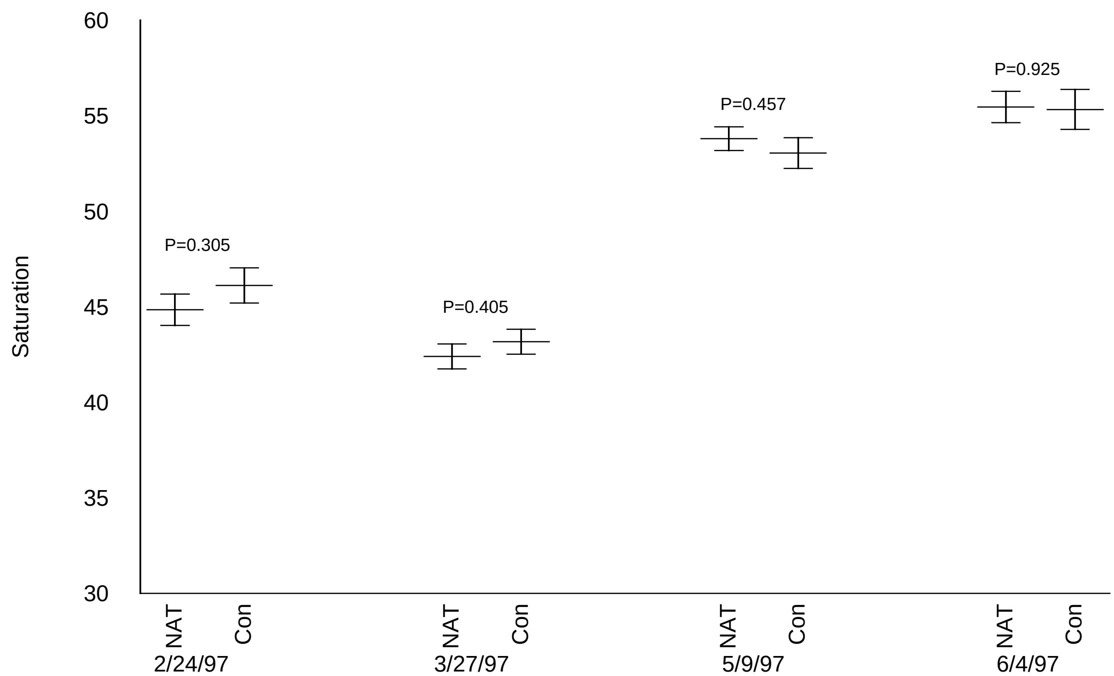

Figure 6. Mean saturation values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1997. P values are based on *t*-tests.

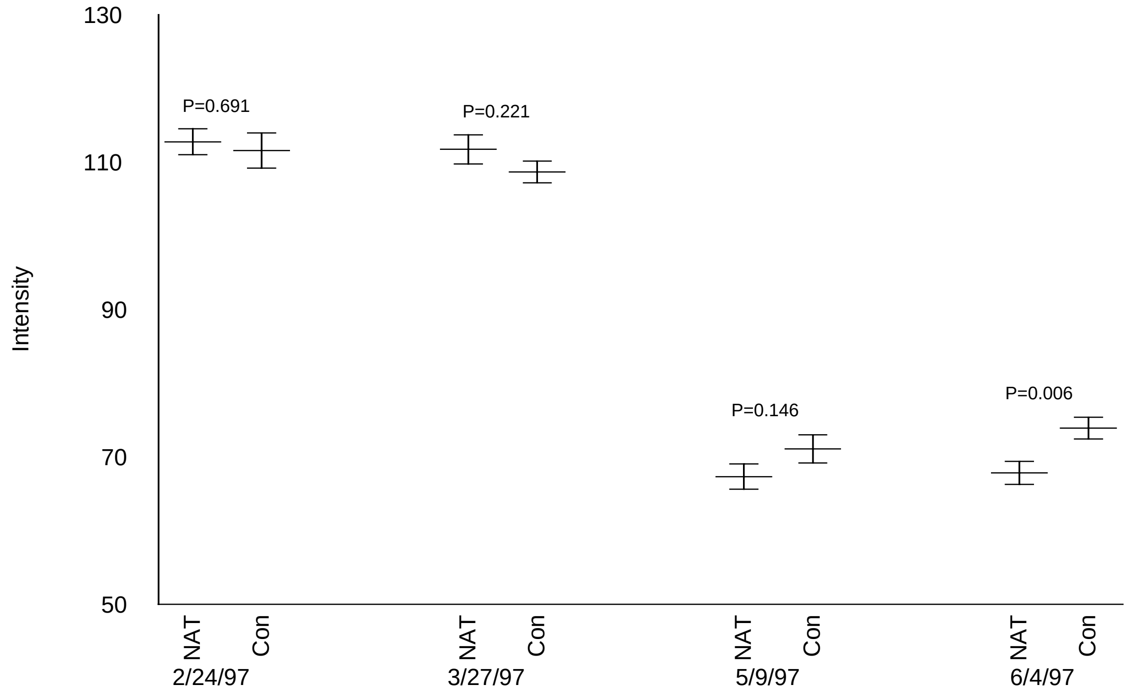

Figure 7. Mean intensity values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1997. P values are based on *t*-tests.

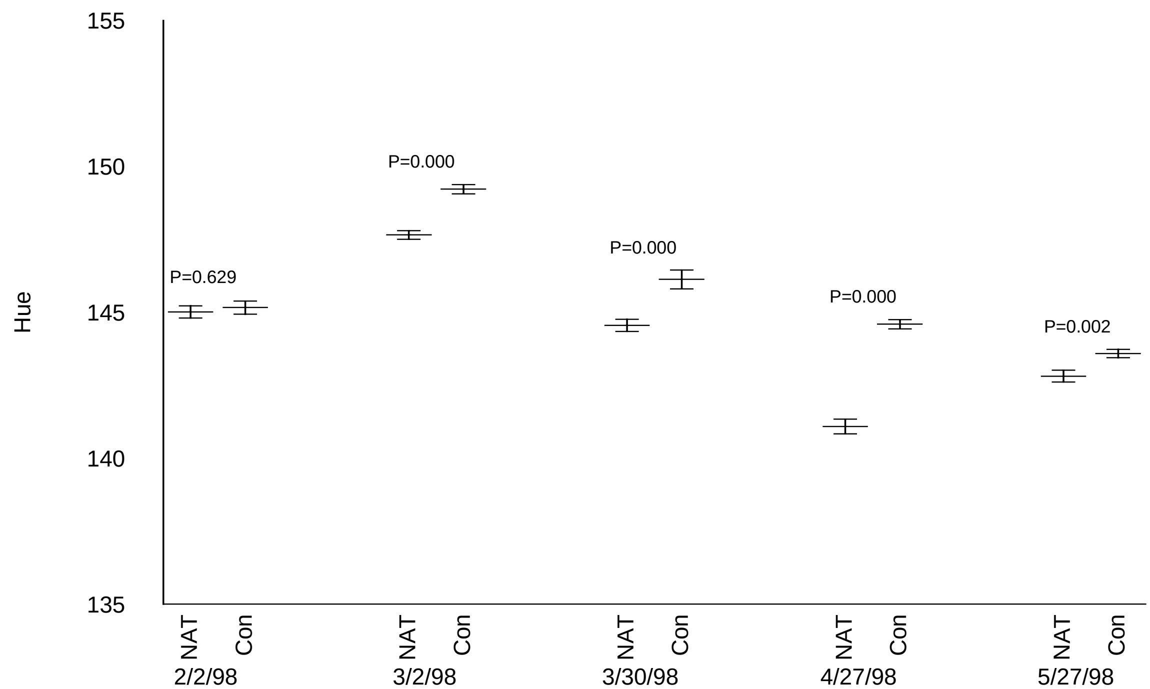

Figure 8. Mean hue values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

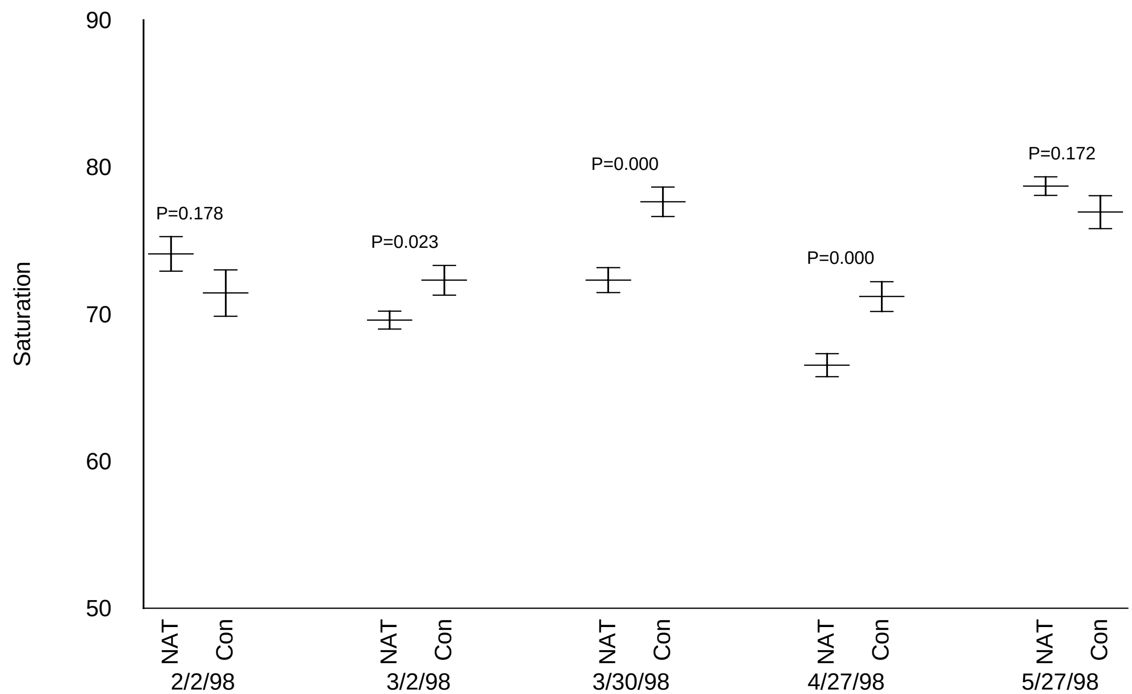

Figure 9. Mean saturation values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

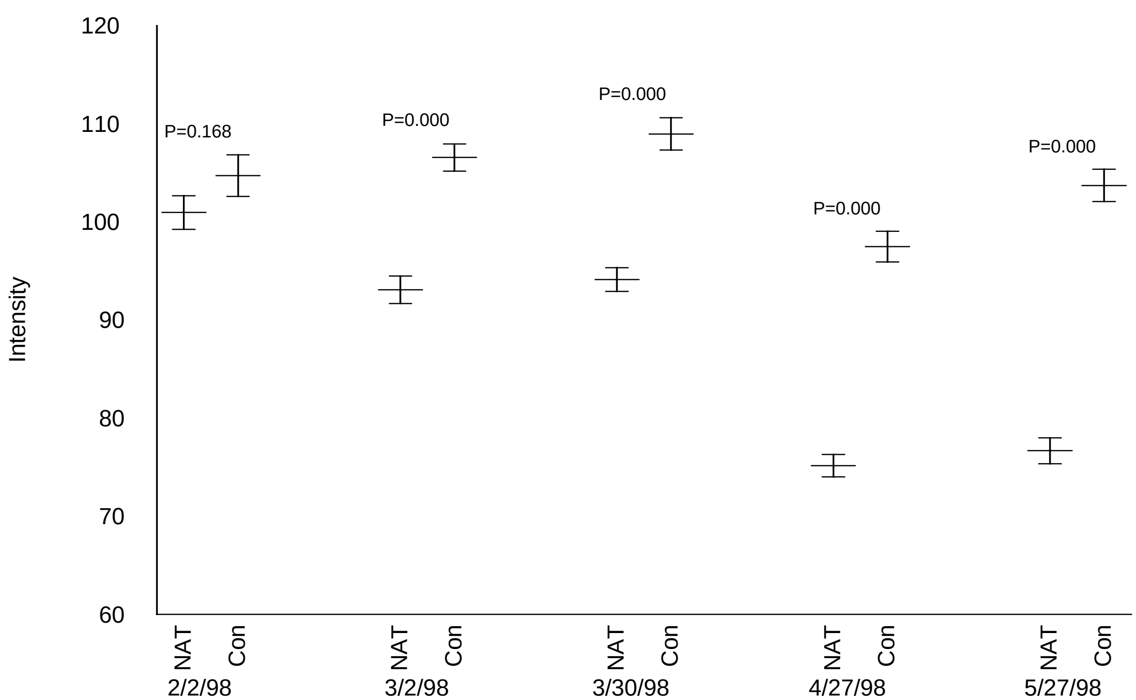

Figure 10. Mean intensity values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

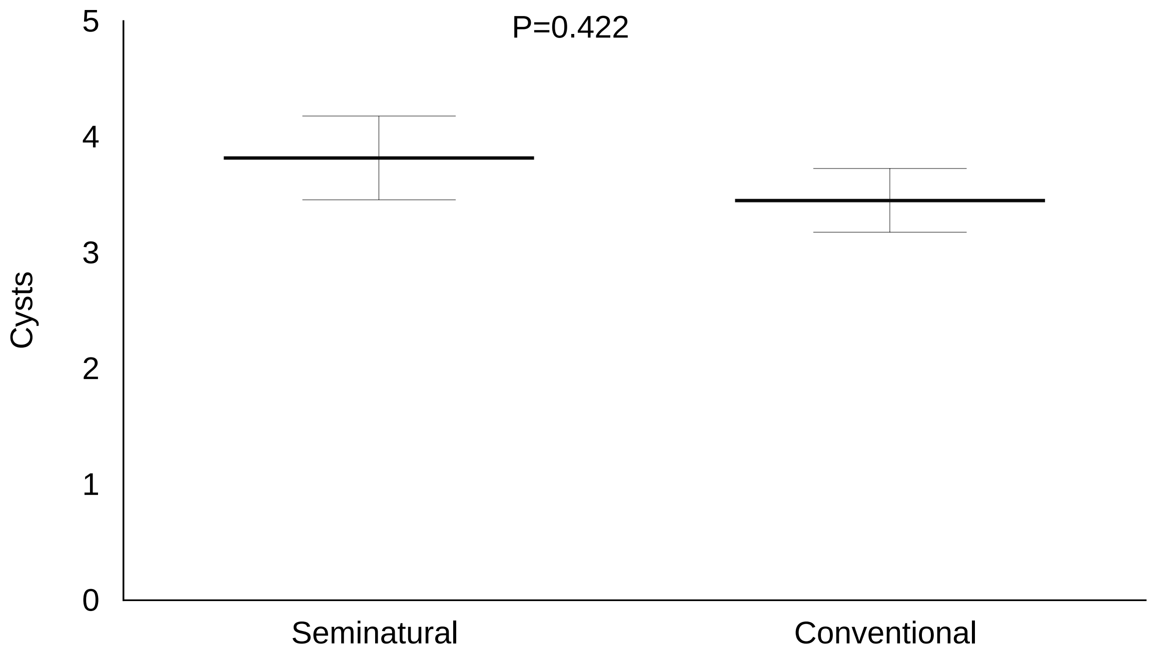

Figure 11. Mean number of *Nanophyetus salmincola* cysts found in kidney smears of fall chinook salmon reared in seminatural (n = 60) or conventional (n = 60) raceways at Forks Creek Hatchery in 1997. P values are based on *t*-tests.

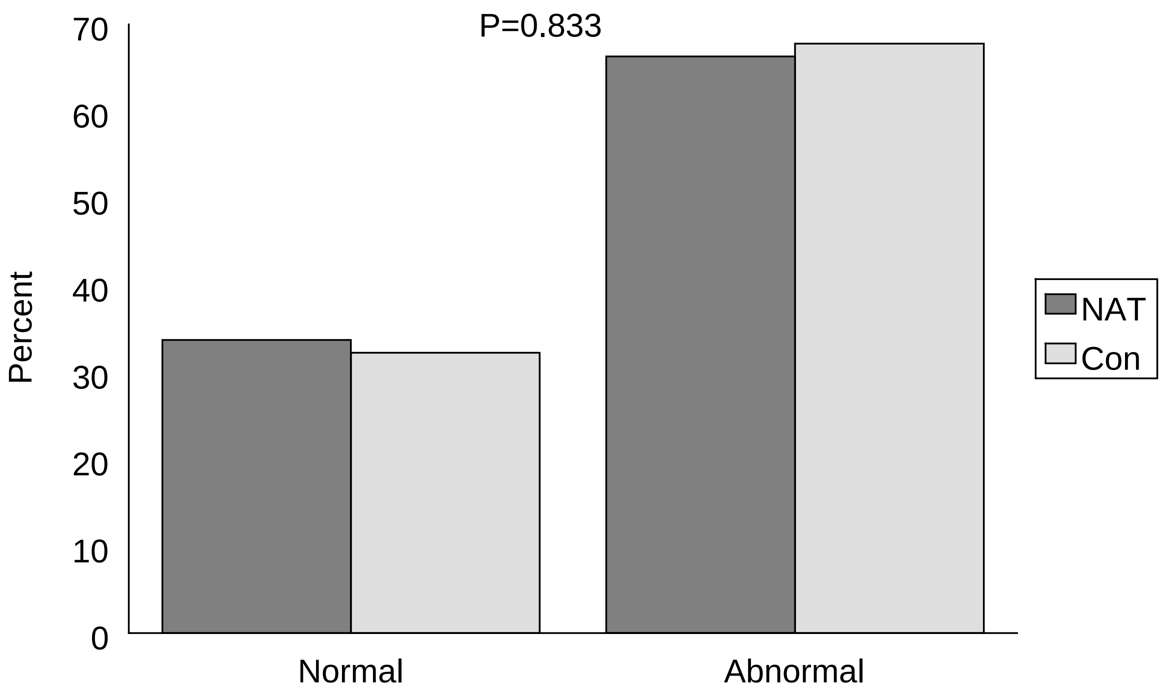

Figure 12. Percentage of fall chinook salmon with normal vs. abnormal fin conditions. Fish were reared in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

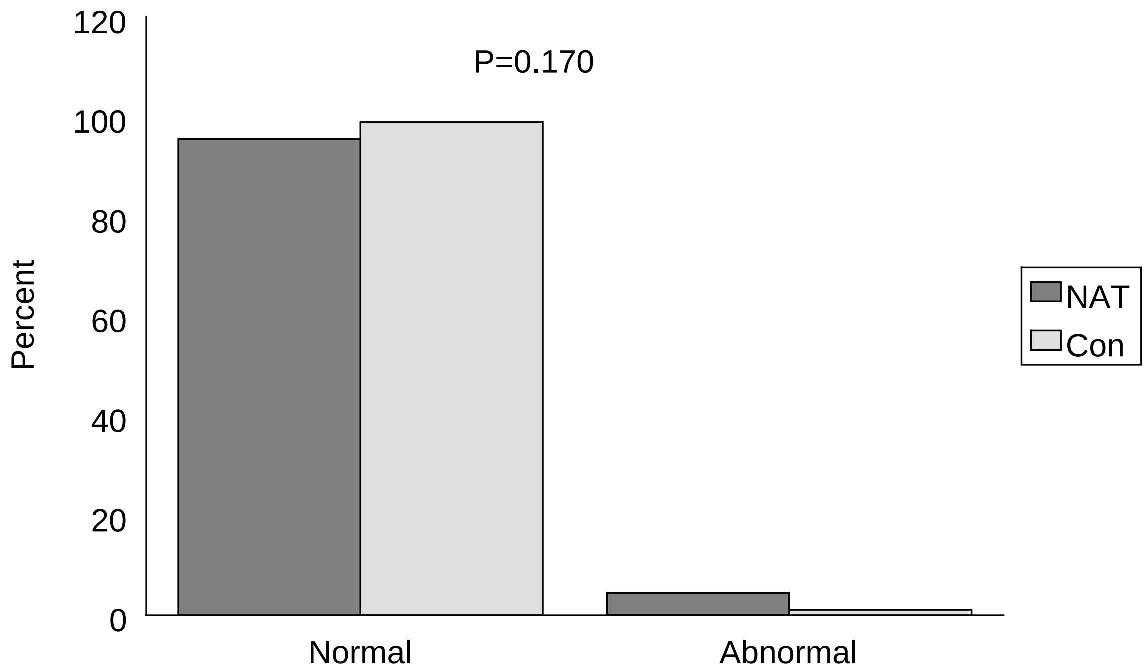

Figure 13. Percentage of fall chinook salmon with normal vs. abnormal kidneys. Fish were reared in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

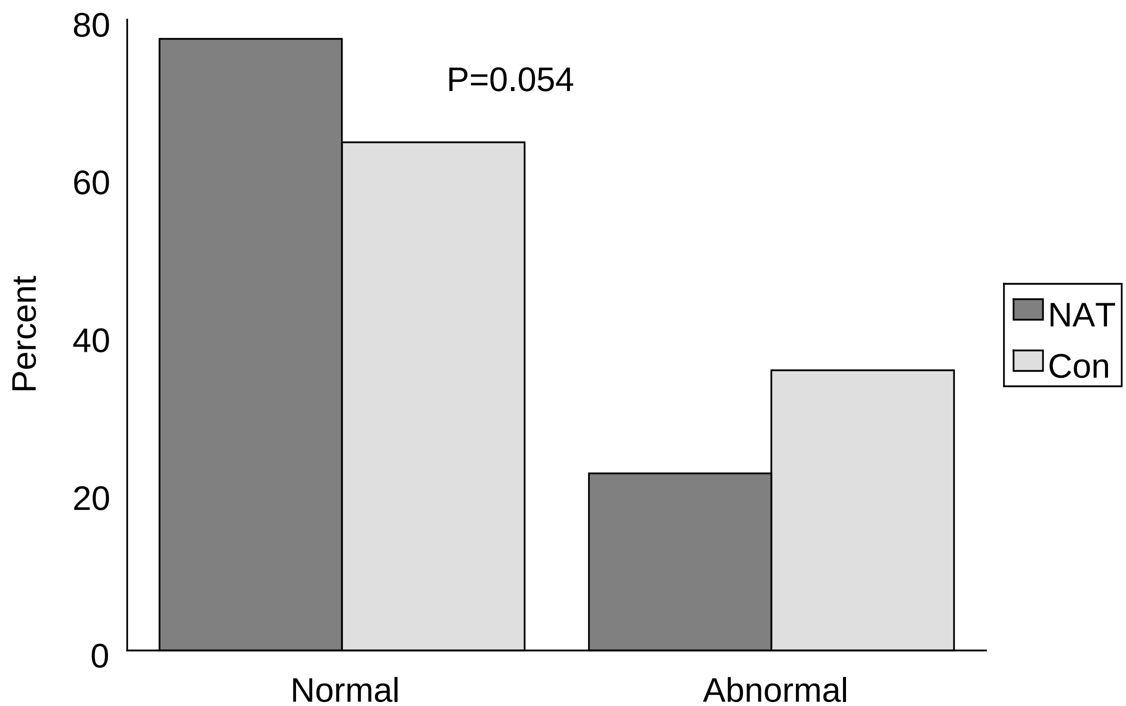

Figure 14. Percentage of fall chinook salmon with normal vs. abnormal spleens. Fish were reared in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

developing in the diseased raceway. In 1998, the fish were checked for *I. necator* and given a formalin bath just prior to release.

The average travel time to the weir for the groups released in 1997 ranged from 1.8 to 3.9 days (Fig. 15). Rearing treatment was not a significant factor affecting the time it took fish to reach the weir, but release date was a significant factor which affected travel time. Fish in the second release generally reached the weir more rapidly than fish in the first release. There was no significant interaction between treatment and release date in the 1997 experiment.

In 1998 fish took longer time to reach the weir, with the average travel time for the groups ranging from 2.96 to 6.49 days (Fig. 16). However, as in the previous year, there was no significant effect of rearing treatment on travel time, but there was again a statistically significant effect of release date on travel time. Fish in the first release took the longest time to reach the weir, fish in the second release took a slightly shorter time, and the fish in the third release reached the weir in the least time. Again there was no statistically significant interaction between rearing treatment and release date.

The majority of the fish released in both 1997 and 1998 survived downstream migration to the weir at Forks Creek Hatchery (Figs. 17 and 18). In both 1997 releases, slightly more seminaturally-reared than conventionally-reared chinook salmon were recovered at the weir, but the difference was not statistically significant. In two of the three 1998 releases, significantly more seminatural than conventional fish were recovered at the weir. The other 1998 release also had more seminatural than conventional fish recovered, but the difference was not statistically significant.

In 1997, more than 45,000 coded wire tagged fish were released from each raceway (Table 1) for a total of more than 95,000 fish released per treatment. In 1998, more than 32,000 fish were released from each raceway, for a total of more than 96,000 fish being released per treatment.

Table 1. Coded wire tagged releases of fall chinook salmon from Forks Creek Hatchery NATURES project, 1997-1998.

	Raceway 21	Raceway 22	Raceway 23	Raceway 24	Raceway 25	Raceway 26
Treatment	control	seminatural	control	seminatural	control	seminatural
1997						
CWT + Ad clip	44,172	46,258	47,589	45,368	N/A	N/A
CWT only	2,068	2,235	1,405	1,773	N/A	N/A
1998						
CWT + Ad clip	33,154	34,008	33,267	32,507	32,606	33,994
CWT only	194	0	67	66	97	34

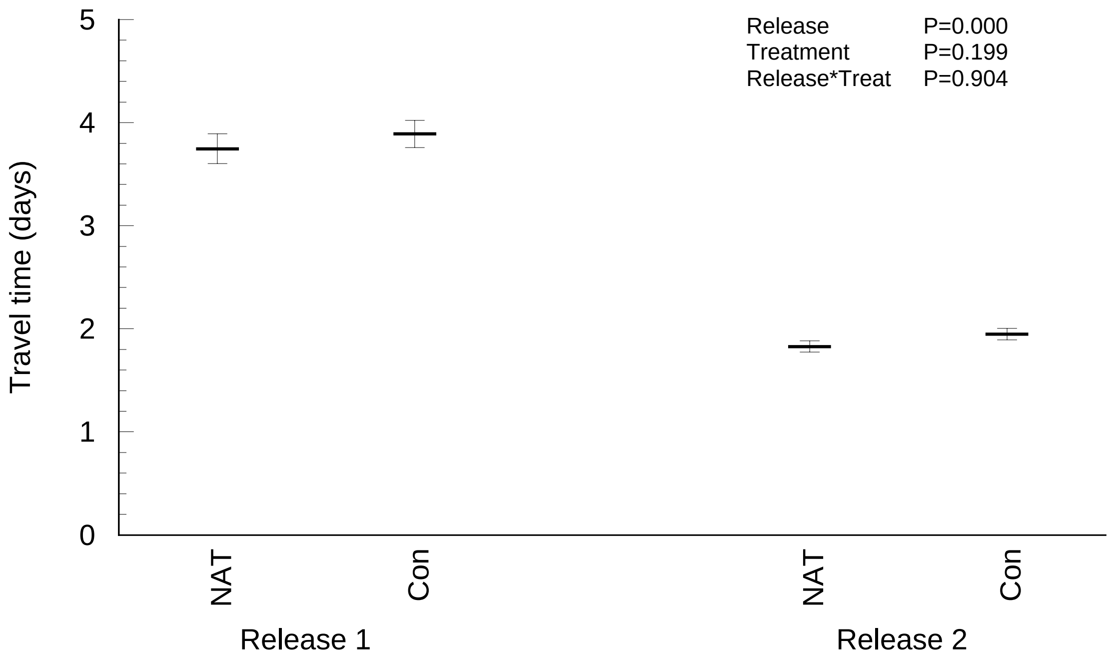

Figure 15. Mean travel time (with standard error bars) for outmigrating fall chinook salmon reared in seminatural (NAT, n = 1,042) or conventional (con, n = 1,036) raceways at Forks Creek Hatchery in 1997. Travel time is measured as days from release above hatchery to recapture at a weir downstream. P values are based on two-factor ANOVA.

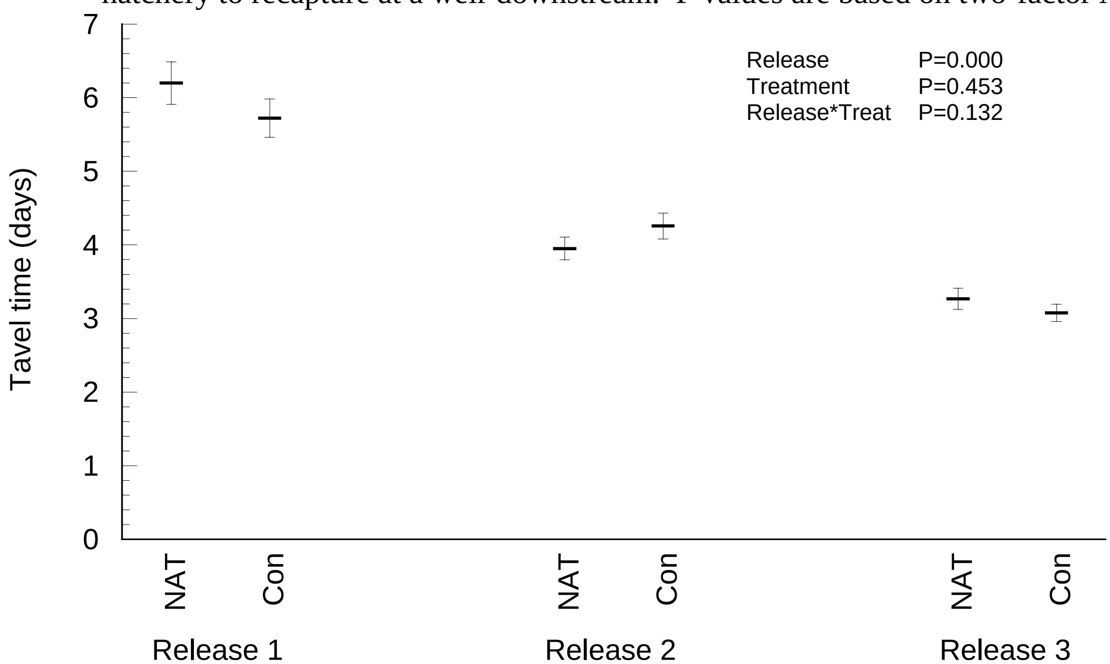

Figure 16. Mean travel time (with standard error bars) for outmigrating fall chinook salmon reared in seminatural (NAT, n = 988) or conventional (con, n = 890) raceways at Forks Creek Hatchery in 1998. Travel time is measured as days from release above hatchery to recapture at a weir downstream. P values are based on two-factor ANOVA.

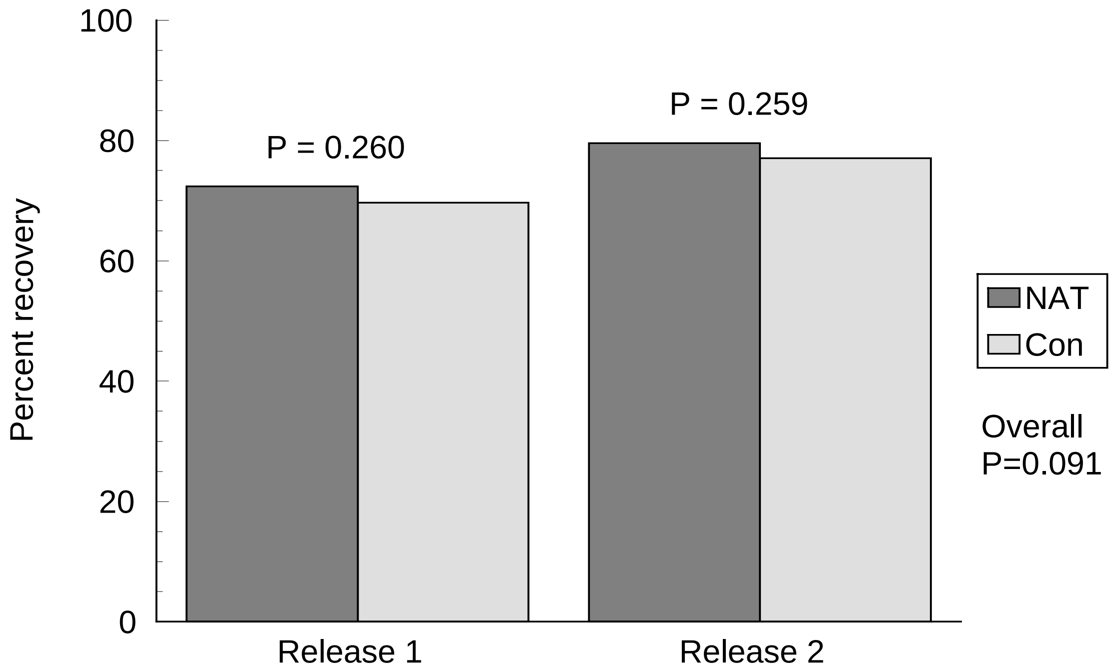

Figure 17. Percent of fall chinook salmon recovered in smolt-to-smolt survival evaluations. Fish were reared in seminatural (NAT, n = 1,042) or conventional (con, n = 1,036) raceways at Forks Creek Hatchery in 1997. P values are based on chi-square analysis.

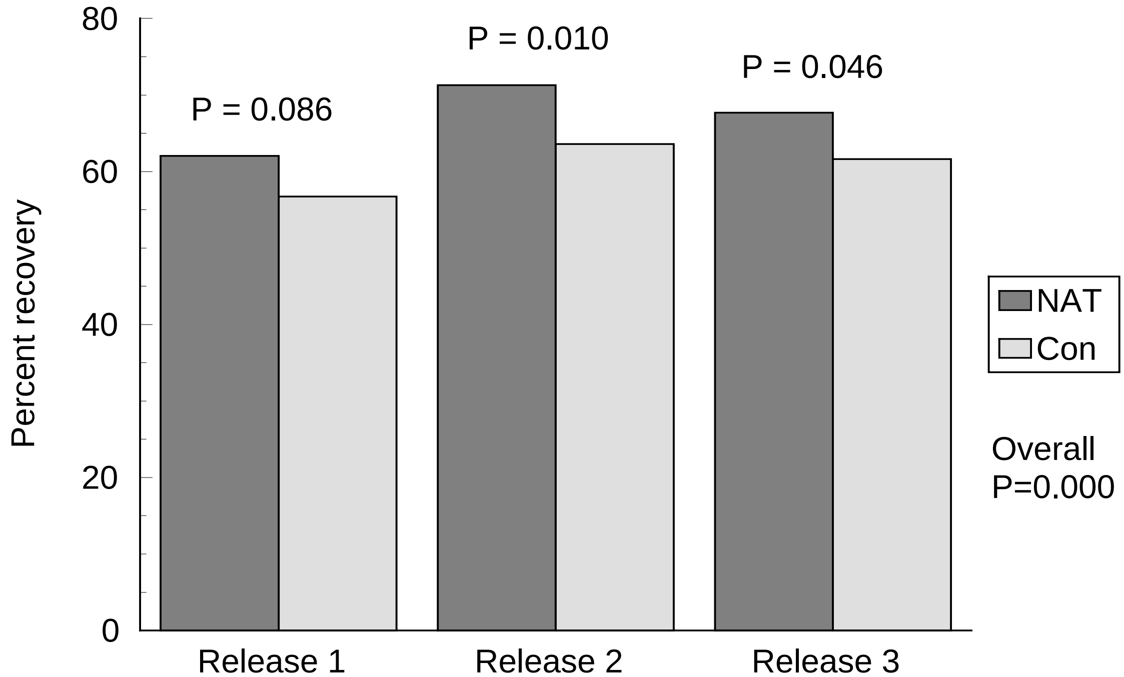

Figure 18. Percent of fall chinook salmon recovered in smolt-to-smolt survival evaluations. Fish were reared in seminatural (NAT, n = 1,001) or conventional (con, n = 899) raceways at Forks Creek Hatchery in 1998. P values are based on chi-square analysis.

Discussion

The recovery trend in all five paired releases supports the work of previous studies (Maynard et al. 1996 b, c, d) that rearing fish in a seminatural raceway habitat tends to improve in-stream survival. The skin color of the fish reared in the two treatments was different, reinforcing the earlier hypothesis that the development of proper camouflage coloration for the postrelease environment is responsible for the increased in-stream survival (Maynard et al. 1996c, 1996d). In nature, this attribute would benefit the fish not only as they move down the river, but also as they reside for several weeks over the dark brown substrate of the Willapa estuary.

The small differences in in-stream survival in 1997, which were not statistically different, may have been the result of poorly chosen or unusual experimental criteria that year. First, the walls in the control raceway were colored dark gray, which would promote the development of darker skin coloration in both treatments. Second, the continuous sedimentation in the conventional raceways that occurred during the first 2 months of that year produced a skin coloration in both treatments which blended with the natural brown background of the Willapa River. Finally, the resin of the original pavers discolored, perhaps resulting in lightened skin coloration of the fish reared in seminatural raceway habitat that year.

In 1998, the differences in hue, saturation, and intensity values between the two treatments were more consistent than in 1997, and the relative in-stream survival advantage for seminaturally-reared fish was greater. These observations, coupled with the increased in-stream survival in 1998, suggest that lightening the color of the walls of the control raceways, and replacing the pavers, were important improvements. The reduced sedimentation in 1998 was probably another critical factor increasing the relative survival differences.

In the Forks Creek experiment, the loose gravel substrate used in three earlier seminatural raceway habitat studies (Maynard et al. 1996a, b, d) was replaced with resin rock pavers to reduce the labor involved in management. Although not as easy to work as the conventional raceways, the pavers were much easier to vacuum than the loose gravel. Once modified, the new pavers provided similar color development and survival benefits previously found with loose gravel.

The modified pavers also provided a similar or even more hygienic rearing environment than conventional rearing habitat, as disease broke out more in the conventional tanks rather than in the seminatural tanks. In the 1992 experiment (Maynard et al. 1996d), this increased hygiene was attributed to the undergravel filters removing decaying particulates (food and feces) from the water column. Possibly, as food falls in the interstitial spaces between the pavers, they too keep it from being put back into the water column. At this point in time, the main improvement to the pavers would be to make them even easier to vacuum by using smaller rocks, similar in size to those used for exposed aggregate. This smaller rock would enable the wheels of the vacuum to move easily, and bring the vacuum head closer to the substrate. On the other hand, fewer but larger rocks might be easier to vacuum and therefore some further designs still need to be engineered and tested.

The type of habitat in which fish are reared does not appear to affect their travel time downstream. However, in both 1997 and 1998, the release time had a significant effect on travel time. The earlier the fish were released, the longer their mean travel time. It is possible that later released fish were more advanced smolts with a more urgent need to migrate downstream. It is also possible that the stacking of several releases confounds travel time results. In every release in 1997 and 1998, a very large percentage of fish moved immediately downstream, and was recovered at the weir within 3-5 days of release. After this, the remaining fish trickle through in smaller steady quantities. When a second group is released “on top” of the previous release group, a secondary spike of recoveries of the first release corresponds with this initial spike of recoveries from the second release group. These fish flushed out from the first release by the second release might consist of fish which would ordinarily reside in the creek longer. In either case the flushed fish in the earlier release group lengthen the group’s mean travel time, while the greater number of fish in the later release group that cannot take up residence decrease mean travel time. Fish were not pushed out of the creek more rapidly in later releases by floods or the current, as water flow in the creek decreased over time. Thus later releases may have shorter travel times because:

(i) they are better developed smolts,
(ii) mean times are lowered by the displacement of earlier residents and later released fish being less likely to obtain resident sites, or
(iii) other factors that may have produced this travel time pattern.

At this point the authors believe it is more probable that the second factor (ii) is responsible for the decreased travel time observed for the later releases in 1997 and 1998.

The preliminary results indicate that seminatural raceway habitat rearing improves cryptic coloration and in-stream survival in production size vessels and at production densities. The majority of the study fish are now at sea. As these fish return over the next several years, they should provide answers to the question of whether or not seminatural rearing also improves smolt-to-adult survival.

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Section 8

CHEMICAL ALARM SIGNALS AND COMPLEX HATCHERY REARING HABITATS AFFECT ANTIPREDATOR BEHAVIOR AND SURVIVAL OF JUVENILES OF CHINOOK SALMON (*O. TSHAWYTSCHA*)[3]

by

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Introduction

Antipredator conditioning and the addition of seminatural habitat to rearing vessels are among numerous culture techniques reviewed by Maynard et al. (1995) in attempts to maximize postrelease survival of hatchery-reared anadromous salmonids. Predator avoidance differences in hatchery and wild juvenile steelhead (*O. mykiss*) have a genetic basis (Johnsson and Abrahams 1991, Berejikian 1995); however, within populations, the ability of hatchery-reared juvenile salmonids to avoid predation improves with experience. Laboratory studies have demonstrated that antipredator behavior and predator avoidance by juveniles of several salmon species increased after exposure to predators (Patten 1977, Olla and Davis 1989, Berejikian 1995, Healey and Reinhardt 1995), and this is the necessary stimulus to trigger innate antipredator responses (Suboski 1988, Olla and Davis 1989). However, in-culture predator training has had limited success improving survival of juvenile salmonids released into natural streams (Thompson 1966, Kanayama 1968). The efficacy of antipredator training programs in existing hatcheries will depend most probably on their ability to improve postrelease survival significantly, as well as their cost and application.

Numerous non-salmonid fish species communicate danger through chemical alarm substances in the skin (reviewed by Smith 1992). Chemical alarm signaling has also been demonstrated with rainbow trout (Brown and Smith 1997, Brown and Smith in press), and suggests that chemical signals may be applied to condition naïve hatchery salmonids to recognize and avoid predators after release. The chemical substances which elicit fright responses by conspecifics are most commonly produced in epidermal cells. Damage to these cells caused by a predator attack releases the alarm substance, which warns nearby conspecifics of possible danger and elicits fright responses (Smith 1992).

Some species, including rainbow trout, learn to associate predator odor with perceived danger, and subsequently respond to predator odor alone (Magurran 1989, Chivers and Smith 1994, Chivers et al. 1995, Brown and Smith in press). This transfer of fright response to a neutral stimulus (predator odor) is known as releaser-induced recognition learning (Suboski 1990). If acquired predator recognition could be learned by treating fish with paired alarm signals in the hatchery, both predator detection and ultimately survival might be improved after release into natural streams.

Alternative hatchery rearing technologies may also be used to improve postrelease survival of juvenile salmon. The combination of natural substrates, submerged structure, overhead cover, and underwater feed delivery systems added to hatchery rearing vessels improves in-stream postrelease survival of smolts of chinook salmon more than conventional (i.e., barren) environments (Maynard et al. 1996a, 1996b). Differences in post-release survival between juveniles reared in 'complex' environments compared with barren vessels often occur within 14 days after release, suggesting that differential predation is responsible for differences in survival. The benefits of such complex rearing environments probably include morphological color changes (Fujii 1993) which improve cryptic coloration. This reduces the probability of detection and attack by predators.

The purpose of the study was first to determine whether chinook salmon possess a chemical alarm signaling mechanism, and whether they acquire predator recognition on the basis of paired alarm signals. Secondly, whether seminatural habitats and paired chemical alarm signals affect vulnerability to predation and postrelease survival in a natural stream.

Methods

Study Population and Culture Protocols

Juvenile chinook salmon were obtained from the WDFW hatchery at Bingham Creek. Bingham Creek is a tributary to the East Fork Satsop River, which flows into the Chehalis River and eventually the Pacific Ocean at Grays Harbor, WA. A total of 1,100 emergent fry were stocked in each of nine rearing vessels (1.8 m diameter) at the hatchery on 28 February 1997. They were fed a standard commercial diet several times a day, five days a week. Fish from this Bingham Creek population typically undergo smoltification between late June and early July.

Two different rearing treatments ('complex' and 'barren') were used in the experiment.
(i) Complex treatment: Four rearing vessels were fitted with a rugose substrate, in-water structure, overhead cover, and an underwater feed delivery system in an attempt to mimic a natural stream environment. The substrate was oval-shaped gravel (2-3 cm diameter) cast in a non-toxic fiberglass resin. The in-water structure was plastic camouflage net (brown and green) hung on rectangular PVC frames (0.69 × 1.29 m) leaning against the center drain pipe at a 45^{o} angle. Feed was carried through a PVC tube (7.5 cm diameter) by water (6 L/minute) into two smaller plastic tubes (2.5 cm diameter) and delivered about 20 cm beneath the water surface (42 cm deep). Water was turned on approximately 5 minutes before introducing the feed, and turned off on completion. One half of each tank was covered with a double layer of camouflage net; one quarter was covered with a single layer of net, and the remaining quarter was uncovered, except for bird netting.
(ii) Barren treatment: Four tanks contained no additional substrate, structure, or overhead cover (except bird netting). The fish were hand-fed at the same time intervals as those in the complex tanks.

The position of the eight tanks was randomized to reduce any bias in fish behavior and survival due to location. The ninth tank was barren, and held the fish for Experiment 1 (below).

Experiment 1: Behavior of fish treated in aquaria

A laboratory experiment was made first to determine whether chinook salmon possess a chemical alarm signaling mechanism, and if they could be conditioned in laboratory aquaria to recognize and exhibit a fright response to the odor of predatory cutthroat trout. Eight juveniles were sacrificed to provide the chinook salmon extract (CSE). About 30 cm^2 of skin and muscle tissue were removed in total, and homogenized in distilled water (1,500 ml) before filtering through polyester filter floss. The control extract (STE) was made by the same procedure with swordtails, a species which is phylogenetically distant, allopatric, and possesses no apparent alarm pheromone (Mathis and Smith 1993a). Both extracts were frozen in 50 ml lots.

Cutthroat trout odor (CTO) was produced by holding two fish (245 cm and 295 cm fork length) for 15 hours in an aerated water bath (28 L volume). After the fish were removed the water was frozen in 50 ml lots. The two predators were caught in Bingham Creek. They were first fed non-fish prey (earthworms) for over three weeks to reduce alarm pheromones of prey fish in their chemical odors and feces (Mathis and Smith 1993b, 1993c, Brown et al. 1995).

The experiment was carried out at the NMFS Manchester Research Station. The juvenile salmon were transferred from Bingham Creek on 1 June 1997 and relocated in a single rearing vessel (1.1 m diameter). Laboratory aquaria (170 L capacity) were situated in two indoor flume tank (9.0 × 1.5 m), ten in one flume and five in the other. Each aquarium was opaque on three sides, reducing all visibility between them. The clear side faced a side wall of the flume, which was constructed of double-paned glass for unobstructed viewing. Each aquarium contained a deep layer (3 cm) of gravel (1.0-1.5-cm diameter). The water level was maintained at 25 cm. Fresh water (6 L/minute) was delivered to each aquarium through a funnel and polyvinyl tube which opened mid-water depth at one end. It was exhausted through a double siphon at the other, and then emptied into the flumes to create a water bath around each aquarium, maintaining fairly constant temperatures (12.5-13.0^{o}C). Light was provided by a solid bank of wide-spectrum fluorescent lights on a simulated natural photoperiod cycle (16:8 light/dark). In the center of each aquarium a tile (15 × 15 cm) was placed 5 cm above the gravel and surrounded by a PVC ring (2.5 cm high) to provide both overhead and lateral cover.

One juvenile was placed in each of the fifteen aquaria on 9 June 1997, and fed commercial feed three times daily over the next three days. On 12 June 1997, the fish were each fed approximately 20 pellets between 0745 and 0830 hours. Each trial consisted of eight minute pre-stimulus and eight minute post-stimulus observations. Twenty minutes prior to pre-stimulus observations 2.5 ml of live Daphnia were introduced. At the end of the eight minute pre-stimulus observation, 50 ml of either CSE or STE combined with 50 ml of CTO water were introduced through the water inflow tube. During both pre and post-stimulus observations, data were recorded for: (i) number of food strikes, (ii) time spent in the lower, middle, and upper thirds of the water column, (iii) time spent under cover, and (iv) time spent motionless. The frequency of darting behavior (quick-burst swimming movements) was also recorded, but the behavior was too infrequent to be analyzed.

Two days later, the same fish were tested for their response to CTO alone to test the null hypothesis that no acquired recognition learning occurred as a result of the paired stimulus introduction. These trials were carried out similarly to the paired stimulus trials except that only cutthroat trout water was introduced after the 8 minute pre-stimulus observations. After the trials were complete the fish were removed and held individually in tubes (6 cm diameter × 25 cm long) constructed of plastic screening which allowed water to pass through. The 15 tubes with fish, individually labeled, were partially submerged in a common tank (1.1-m diameter). Four days later they were returned to their original aquarium for two days acclimation before tested again for their response to CTO only.

In summary, each of the 15 fish was tested for (a) its response to a paired stimulus (CSE plus CTO, and STE plus CTO) on June 12 (day 1), (b) CTO only on June 14 (day 3), and (c) CTO only a second time on June 21 (day10). A second series was carried out so that each of the 15 fish were tested for (d) their response to a paired stimulus on June 17 (day 1), (e) CTO only on June 19 (day 3) and CTO again on June 26 (day 10).

Differences between pre- and post-stimulus observations were timed for three behaviors: motionless, under cover, and lower third of water column. For frequency of food strikes, a post-stimulus/pre-stimulus value was calculated from the response variable to correct for individual variation in foraging rates. Differences in response variables between experimental (chinook extract plus cutthroat trout odor) and control trials (either swordtail extract plus cutthroat trout odor, or plus distilled water) were compared by Mann-Whitney-U tests.

Experiment 2: Behavior of fish treated in rearing vessels

The experiment was designed to test the null hypothesis that behavioral fright responses to a neutral stimulus would be the same for smolts treated with a paired alarm signal and those treated with a distilled water control applied directly to the rearing vessels.

Skin and muscle tissue (48 cm^2) of chinook salmon was homogenized in distilled water (1 L), filtered, and diluted (4 L in volume) to make an extract (CSE). Cutthroat trout odor (CTO) was produced by placing two live cutthroat trout (fork lengths 380 mm and 255 mm) in aerated water (12 L) for 5 hours.

On 2 July 1997 two of four barren rearing tanks each received CSE (1 L) and CTO (1 L). The other two tanks received distilled water (2 L) as control. Fifty fish (4-5 g weight) from each tank were then transported in separate permeable plastic totes (40 L) to the NMFS Manchester Research Station. The totes were then placed in a common tank (1.1 L), and the fish were fed daily until the experiment began.

On 7 July, eight aquaria received fish from the tanks receiving the paired alarm signal (CSE plus CTO), and seven aquaria received fish from the controls. After three days of acclimation the fish were tested for their response to CTO using the same protocols described in Experiment 1. A second round of trials began with acclimation on 11 July for eight fish from the control tanks and seven from the tanks receiving the paired alarm signal. In summary, there was a total of 30 trials (15 per treatment). Data were analyzed as in Experiment 1.

Experiment 3: Vulnerability to cutthroat trout predation

This experiment was designed to test the null hypothesis that the introduction of a paired alarm signal in hatchery rearing vessels and structurally complex rearing habitats would not affect vulnerability to predation by cutthroat trout.

Following the same protocols applied in Experiment 2, described above, two of the four complex tanks received a paired alarm signal (CSE plus CTO) on 2 July 1997 and the other two tanks received distilled water as a control. Therefore there were two rearing vessels for each of the following combinations of environment and stimulus: (a) complex - stimulus, (b) complex - control, (c) barren - stimulus, and (d) barren - control.

Each of two parallel raceways (25 × 3.1 m) were supplied with stream water (227 L/min). A single layer of gravel (2.0-5.0 cm diameter) covered the bottom of each. Both were completely covered with netting (2.5 cm square mesh) to exclude all avian predators.

On 5 April 1997, cutthroat trout were caught by hook-and-line from the East Fork Satsop River. Nine (average fork length 302 cm) were introduced into raceway 1, and 8 (308 cm) were introduced into raceway 2. Four small Douglas fir trees were placed in each raceway to provide cover for the cutthroat trout and eventually the chinook salmon smolts. The trout were fed earthworms two or three times per week, and were observed feeding on invertebrate drift entering the raceways with the water supply.

On 3 July, twelve juveniles (4.0-4.9 g) from each of the eight rearing vessels were placed in screened-off sections (5 × 3.5 m) in both raceways containing cutthroat trout. Three hours later the screens separating the fish were removed allowing the juvenile salmon and cutthroat trout to intermix. On 9 July, all surviving juveniles were removed from the sections by either seine net or electrofishing. The thermal marks on the otoliths of the juveniles were decoded (Volk et al. 1994) to identify the origins of those surviving predation.

A 2x2 ANOVA was used to test for differences in the proportion of fish surviving predation (arcsine square root transformation) by cutthroat trout ($\alpha = 0.05$). The main effects were structural rearing environment (complex or barren) and stimulus treatment (paired alarm signal or distilled water control), with individual rearing tanks as the units of replication.

Experiment 4: Survival in a natural stream

The last experiment was designed to test the null hypothesis that neither the introduction of the paired alarm signal in hatchery rearing vessels, nor complex rearing environments, would affect postrelease survival in nature. The migration site was 21 km of Bingham Creek, which contains a healthy population of predatory-sized (fork length above 250 mm) cutthroat trout.

The smolts used in this experiment were sampled from the same eight rearing tanks used in Experiment 3, with two rearing tanks for each of the same four treatment combinations. A total of 800 fish from each tank were transported in an oxygenated tank for a 45 minute journey to Bingham Creek. They were released about 21 km upstream from a 100% efficient fish trap. The trap was checked daily for migrants from 3 July through 17 September. Previous post-release survival studies at Bingham Creek have demonstrated that trap recoveries over this period strongly reflect survival (Schroder et al. unpublished data). After recovery, all smolts were preserved in alcohol and their otoliths decoded to identify their rearing tank and treatment.

A 2x2 ANOVA was used again to test for differences in the proportion fish (arcsine square root transformation) from each tank were recovered at the weir. A Tukey's HSD test was used to test for paired differences among treatments ($\alpha = 0.05$).

Results

Experiment 1: Behavior of fish treated in aquaria

Paired stimulus trials (Day 1)

Smolts receiving a combination of chinook salmon extract and cutthroat trout odor (CSE plus CTO) responded by reducing their foraging activity following the stimulus much more than smolts receiving a combination of swordtail extract and cutthroat trout odor (STE plus CTO) - ($P < 0.01$; Fig. 1a). Fish treated with CSE plus CTO also spent significantly more time in the lower third of the water column ($P = 0.03$; Fig. 1b), and more time motionless ($P < 0.01$; Fig. 1c) after the stimulus was introduced than did STE plus CTO-treated fish. The two groups of fish did not differ in their time spent under cover ($P > 0.50$). In summary, fish receiving conspecific extract demonstrated stronger antipredator responses than those receiving the swordtail extract control.

Cutthroat odor (CTO) trials (Days 3 and 10)

When CTO was provided as a stimulus on day 3 to the same fish that received either the paired alarm substance (CSE plus CTO) or the control substance (STE plus CTO), the CSE-treated fish spent more time motionless than STE-treated fish ($P = 0.02$; Fig. 1a). This suggests that CSE-treated fish learned to associate cutthroat trout odor with danger. There were no significant differences between the two groups for any other behaviors ($P > 0.25$ in all cases). When the same fish were re-tested for their response to cutthroat odor on day, there were no significant differences detected between the STE and CSE groups (Fig. 1a, b, and c).

Experiment 2: Behavior of fish treated in rearing vessels

Fish treated in rearing tanks with either the paired alarm substance (CSE plus CTO) or the distilled water control did not differ in their response to cutthroat trout odor in laboratory aquaria with regard to motionless behavior ($P = 0.17$), number of food strikes ($P = 0.38$), or time spent in the lower third of the water column ($P = 0.22$).

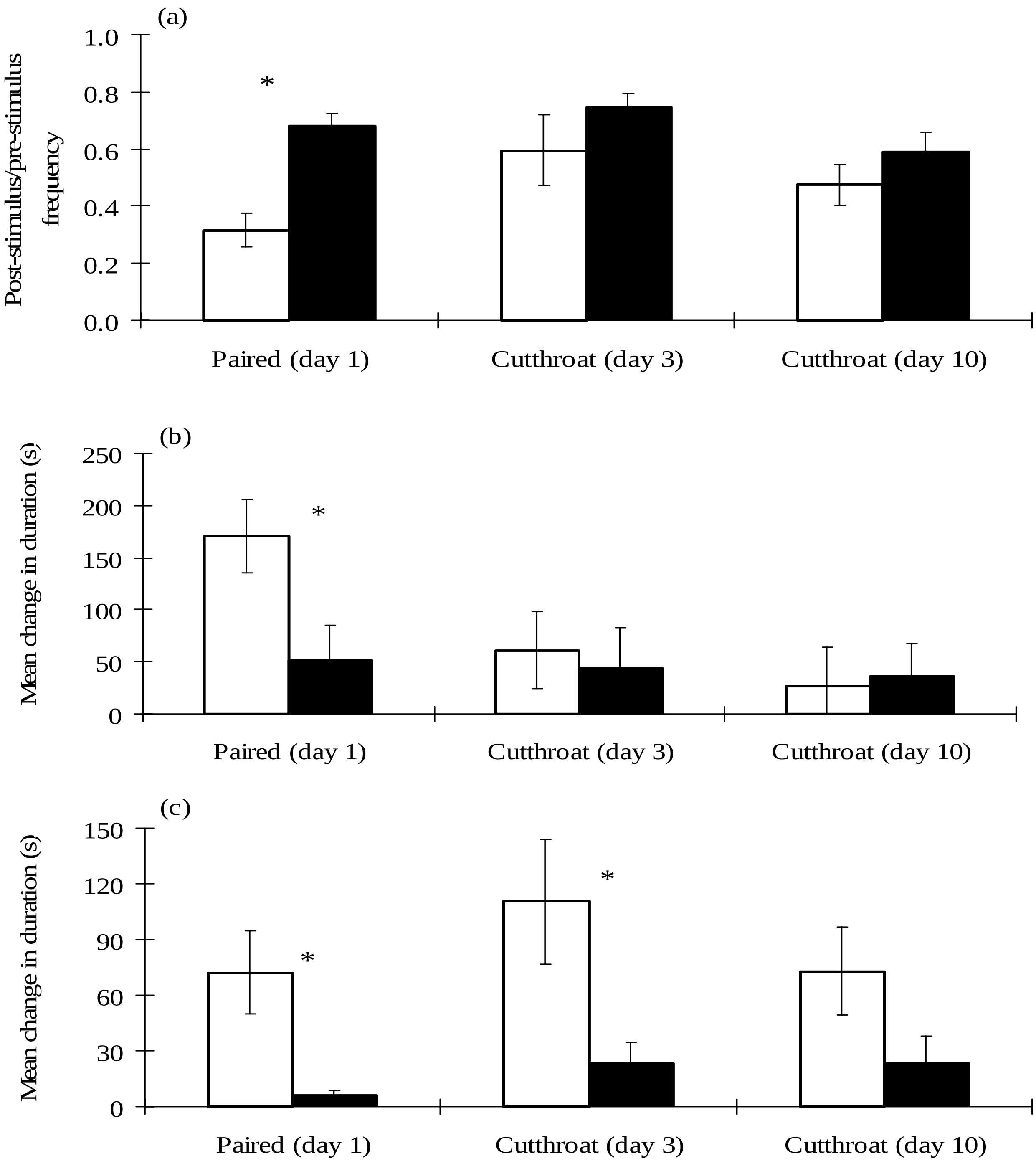

Figure 1. (i) Mean (± s.e) change (post-stimulus divided by pre-stimulus) in ratio of food strikes (panel A); (ii) change (post-stimulus minus pre-stimulus) in time spent in lower third of water column (panel B); and (iii) change (post-stimulus minus pre-stimulus) in time spent motionless (panel C) for smolts treated (a) with chinook salmon extract + cutthroat trout odor, or (b) swordtail extract + cutthroat trout odor on day 1 in laboratory aquaria. Data from trials on days 3 and 10 represent responses of same fish to cutthroat trout odor only.

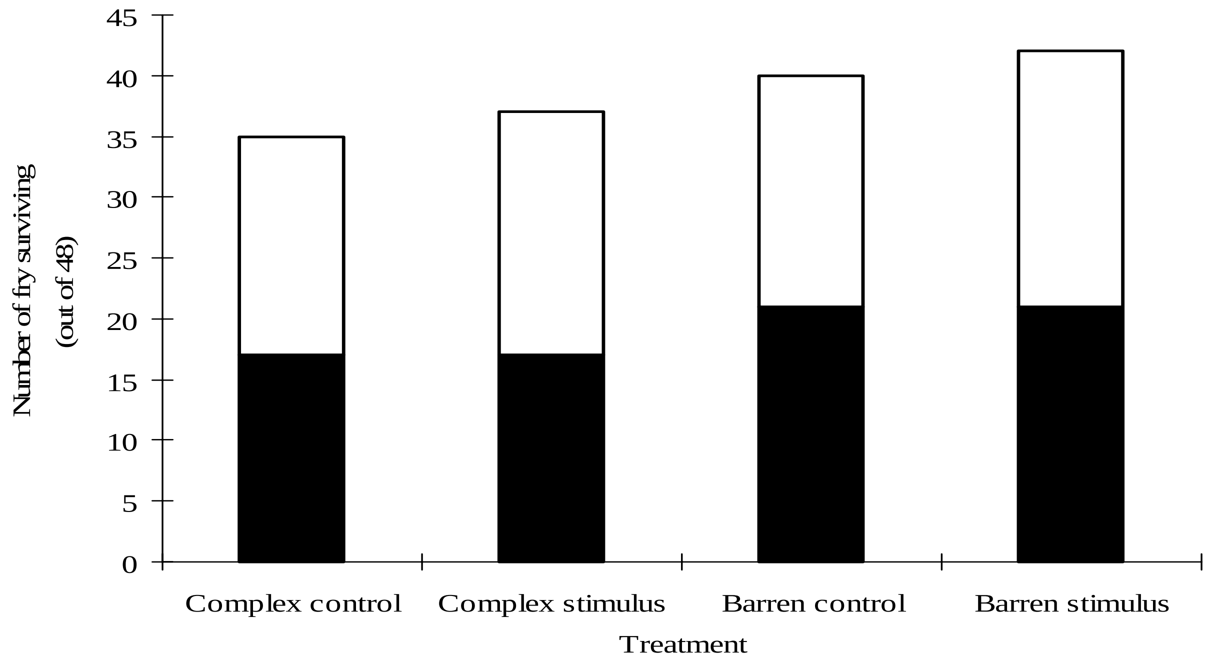

Figure 2. Number of smolts surviving predation by cutthroat trout in two raceways. No significant differences existed among the four treatments.

Experiment 3: Vulnerability to cutthroat trout predation

The proportion of fish from the four rearing treatments combined which survived predation in raceway 1 (79%) was similar to that in raceway 2 (81%). There the data were pooled to test for treatment effects in the two trials combined. There was no significant effect of rearing environment ($P = 0.13$), stimulus introduced ($P = 0.49$), or their interaction ($P > 0.50$), on the proportion of fry surviving six-day exposure to cutthroat trout (Fig. 2).

Experiment 4: Postrelease survival in a natural stream

Of the 6,400 smolts released into Bingham Creek on 3 July 1997, 1,890 (29.5%) were recovered at the weir by 17 September 1997. A significant rearing environment by stimulus interaction existed for the number of smolts recovered ($P = 0.022$). A post-hoc analysis revealed that fish grown in the complex tanks and treated with a distilled water control were recovered at a lower rate than fish from the complex - stimulus ($P = 0.035$), barren - stimulus ($P = 0.021$), and barren - control ($P = 0.014$) treatment combinations (Fig. 3). Differences in recovery rates were evident within one week after fish began entering the trap at the weir (Fig. 4), suggesting that differences in mortality between treatments occurred soon after release.

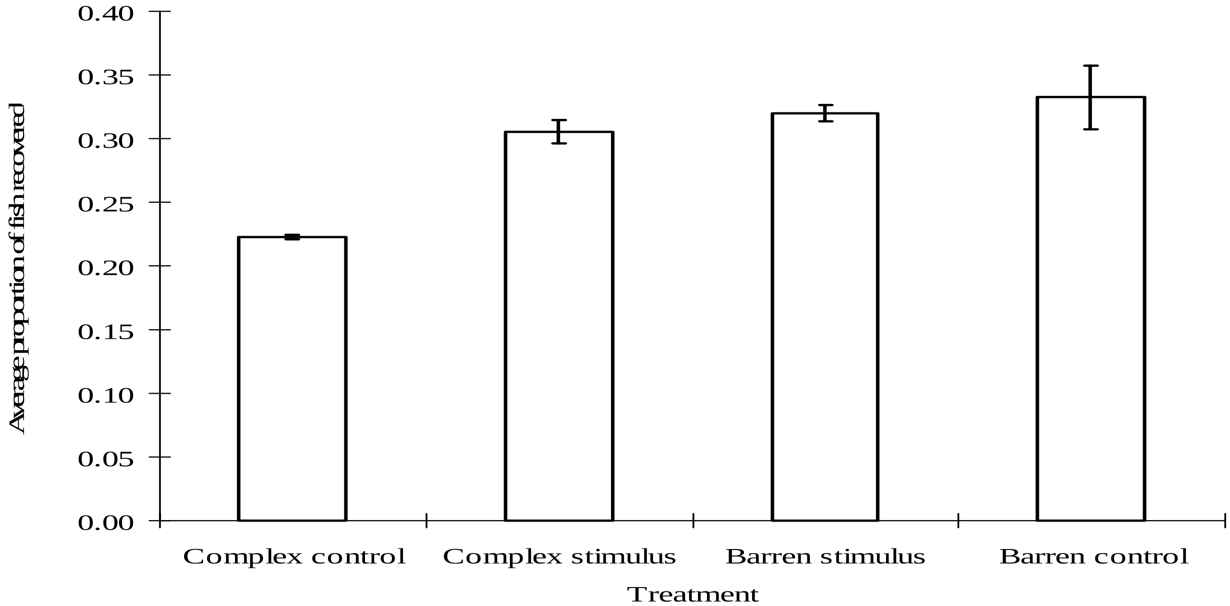

Figure 3. Average proportion of smolts (± s.e.) recovered after release (untransformed data). Smolts from complex - control combination of treatments were recovered at a lower rate (P < 0.05 for each pairwise comparison) than from other treatment combinations (P > 0.50).

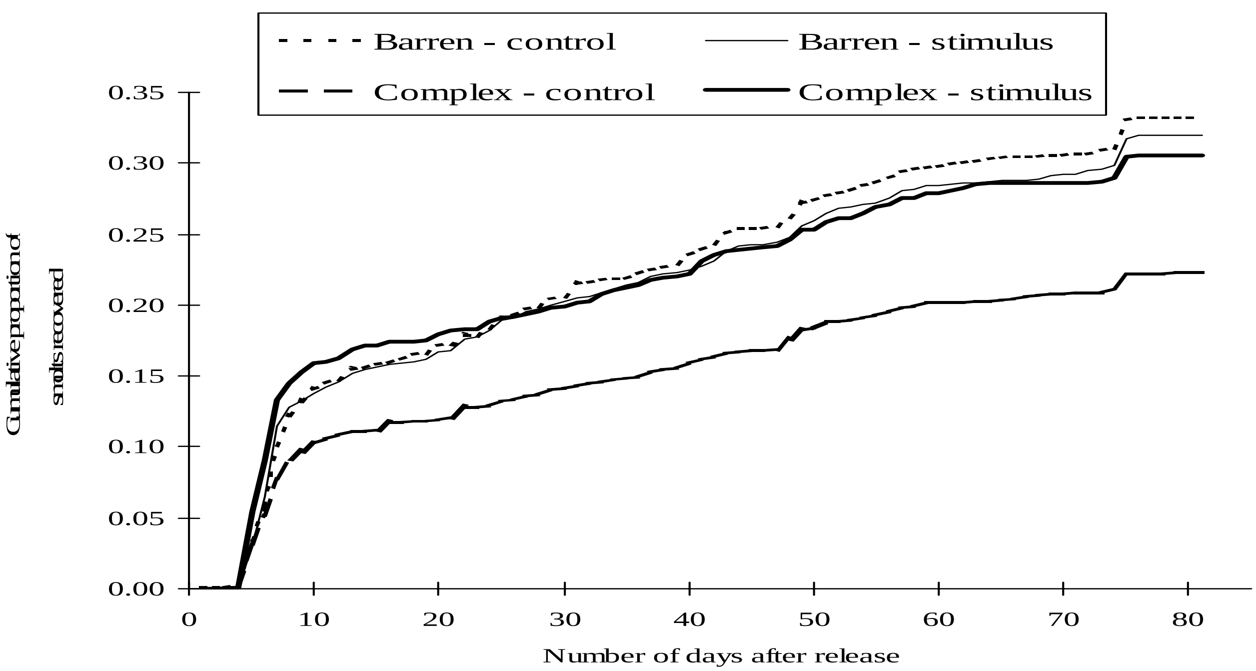

Figure 4. Cumulative daily recoveries of smolts at Bingham Creek weir from four combinations of two rearing treatments (complex and barren) and two stimulus treatments (stimulus and control).

Discussion

In the study, chinook salmon smolts demonstrated the presence of a chemical alarm signaling mechanism by exhibiting much stronger fright responses to combined conspecific extract and a predator odor (CSE plus CTO) than to swordtail extract and predator odor (STE plus CTO). The reduction in feeding, increased time spent motionless, and increased time spent near the substrate are common antipredator responses of salmonids, reducing detection and increasing chances of survival (Olla and Davis 1989, Donnelly and Whoriskey 1993, Gotceitas and Godin 1993). When cutthroat odor was introduced two days later, smolts previously treated with conspecific extract spent more time motionless than those previously treated with swordtail extract.

Healey and Reinhardt (1995) found a reduction in swimming activity was the primary response exhibited by chinook salmon fry to rainbow trout predators. Reduced swimming activity appears to be a general defense against predation for other juvenile salmonids (Lima and Dill 1990, Martel and Dill 1995, Brown and Smith 1997, Brown and Smith in press). It is concluded that the chinook salmon smolts acquired predator recognition; that is, they learned to associate cutthroat trout odor with danger (conspecific skin extract) demonstrated by anti-predator behavior in response to cutthroat trout odor alone. This is the first study to demonstrate chemical conditioning for a congeneric predator. In studies by Brown and Smith (in press), the predators (rainbow trout and Northern pike) were not closely related to the prey.

The acquired predator recognition evident on day 3 of experiment 1 was no longer significant when the same fish were tested for their response to cutthroat odor on day 10, although, the patterns were similar on day 3 and day 10 (Fig. 1a, b, and c). The lack of significant results for the day 10 trials, and the Experiment 2 trials (fish treated in rearing tanks) may be due to their similar long duration (at least 8 days) between initial treatment application and testing. Also, in both experiments fish were first held for several days either in small tubes or small containers. Therefore handling stress associated with close confinement may have interfered with predator recognition, or the duration between initial paired stimulus application and testing exceeded the learning retention period for these fish. Brown and Smith (in press) demonstrated that rainbow trout retained acquired predator recognition for at least 21 days, but the intensity of behavioral responses to predator odor decreased over time.

The efficacy of antipredator conditioning for juvenile salmonids may not require a long learning retention period. Disparity in postrelease survival of wild and hatchery-reared (i.e., predator-naïve) chum salmon has been shown to decrease over time (Kanid'hev et al. 1970). Learning appears to occur quickly in juvenile salmonids, probably after witnessing only a few attacks on conspecifics (Patten 1977, Olla and Davis 1989, Berejikian 1995). Brown and Smith (in press) speculated that the benefit of antipredator conditioning for salmonids may be greatest in the first days following release, after which acquired predator recognition is more likely to occur in the postrelease environment. The cumulative daily recovery data (Fig. 4) from Experiment 4 support this hypothesis. Assuming no difference in travel time from the release point to the weir among treatments (see Maynard et al. 1996b), substantial differences in survival appeared soon after smolts began to enter the weir (Fig. 4).

Postrelease survival of chinook salmon smolts was affected by their hatchery rearing environment (complex or barren), and whether or not they received a paired alarm signal prior to release. Smolts from tanks receiving the complex rearing treatment and no paired alarm signal (distilled water) were recaptured in lesser numbers than fish from tanks treated in any of the other three combinations of rearing environment and chemical stimulus. This suggests the complex treatment had a negative effect on postrelease survival compensated by the addition of the paired alarm stimulus.

The predation bioassay (Experiment 3) showed no significant effect of either factor, but data were consistent with findings of the postrelease survival study (Figs. 3 and 4). One possible explanation for interaction between the two main factors is that fish grown in tanks containing submerged structure and overhead cover developed a preference for submerged or overhead cover. Hence, they may have made more use of such habitat after release into Bingham Creek than fish grown in barren tanks. Recent work by R. Barrows (U. S. Fish and Wildlife Service, Bozeman, Montana, personal communication) demonstrated that rainbow trout grown in partially covered vessels, preferred covered areas after release into a novel environment more so than fish reared without overhead cover.

An acquired affinity for cover might have put the complex-reared fish in this study at greater risk of capture by cutthroat trout, which prefer stream microhabitats that include abundant structure and cover (Bisson et al. 1988). In the experimental raceways, the cutthroat trout were strongly associated with the submerged conifer structure. Fish receiving the paired alarm substance may have recognized and responded more to the presence of cutthroat trout, thereby reducing the risk of capture. Nonetheless, data presented here are the first to demonstrate a significant effect of conditioning with paired alarm signals on postrelease survival of salmonids.

The addition of complex structure to rectangular rearing vessels has improved the in-stream postrelease survival of chinook salmon smolts in other studies, including a study in Bingham Creek (Maynard et al. 1996a, 1996b). This study found only a negative effect of complex rearing habitat on postrelease survival. Gravel substrates and submerged structure (e.g., denuded conifers) applied in Maynard et al. (1996a, 1996b) were attributed with morphological color changes (Fujii 1993), resulting in induced improved cryptic coloration.

The positive effect of the complex rearing environment in that study, and the negative effect in this study, is difficult to explain. The complex environments used by Maynard et al. (1996a, 1996b) were rectangular, with loose gravel substrate, submerged denuded conifers, and underwater feeders which introduced food from the bottom of the tank. This study had smaller circular vessels, gravel cast in resin for substrate, submerged camouflage netting, and feeders which introduced the food from the mid-water column. These rearing differences, or possible changes in predator types (avian versus piscine), and abundance of predator populations in the intervening three years may have, in some way, accounted for different results.

In summary, chinook salmon smolts appear to possess a chemical alarm signaling mechanism, and the ability to acquire predator recognition on the basis of chemical stimuli. The efficacy of treating hatchery vessels with paired alarm signals did not produce significant results in all experiments. Postrelease survival of smolts reared in complex habitats was increased by the addition of a paired alarm signal, but no differences in the vulnerability to cutthroat trout in experimental raceways were detected.

The results of the experiments with fish treated in rearing vessels should be considered preliminary until further experiments are conducted with a greater number of replicate tanks per treatment. Nevertheless, the potential for using chemically-based antipredator conditioning to improve postrelease survival of smolts of chinook salmon appears promising.

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Section 9

SOCIAL DOMINANCE, GROWTH, AND HABITAT USE OF AGE-0 STEELHEAD (*O. mykiss*) IN ENRICHED AND CONVENTIONAL HATCHERY ENVIRONMENTS

by

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Introduction

Few programs for reintroduction of captive-bred terrestrial and aquatic animals have established wild populations successfully. The lack of success has frequently been attributed to behavioral deficiencies in released animals (DeBlieu 1991, Gipps 1991, Minckley and Deacon 1991, Clark et al. 1994, Olney et al. 1994). Recent listings of Pacific salmon as 'Threatened' or 'Endangered' under the US Endangered Species Act have prompted recovery programs which include captive propagation as a major component (Flagg et al. 1995).

Efforts to improve survival of smolts of chinook salmon released from hatcheries include structural modifications to hatchery rearing vessels intended to provide 'enriched' environments more similar to those in natural streams. Such modifications may have combinations of underwater feed-delivery systems, submerged structure, overhead shade cover, and gravel substrates, or any subset (Maynard et al. 1995). Enriched rearing environments have improved in-stream survival of chinook salmon smolts during seaward migrations in some studies (Maynard et al. 1996), but not all (Berejikian et al. 1999). The concept aims in part to promote development of more natural behavior patterns (Maynard et al. 1995), but so far no behavioral differences between juvenile salmonids grown in 'conventional' and enriched habitats have been identified.

Fitness related behavioral attributes of salmonids can be influenced by environmental factors. Manipulation of environmental factors during culture, such as (i) fish density (Fenderson et al. 1968, Berejikian et al. 1996), (ii) food ration (Symons 1968, Ryer and Olla 1991, Berejikian et al. 1996), and (iii) method of food introduction (Ryer and Olla 1995), have all been shown to influence social behavior. Differences also exist between juvenile salmonids grown in hatcheries and streams for attributes such as agonistic behavior (Berejikian et al. 1996) and microhabitat use (Dickson and MacCrimmon 1982). This obviates the need to modify culture techniques to minimize developmental (and possibly evolutionary) divergence between artificially propagated and wild populations.

Steelhead along the west coast of United States and Canada reside in streams for 1 to 4 (typically 2 or 3) years before migrating to sea. Hatchery programs designed to augment harvest rear steelhead to the smolt stage in 1 year, and release them. However, conservation hatcheries designed to re-establish self-sustaining populations of other Pacific salmon species, such as sockeye, *O. nerka*, have opted for a variety of reintroduction strategies which involve releases at various life history stages (Flagg et al. 1995). Recent listings of some steelhead populations under the U.S. Endangered Species Act present the likelihood that various release strategies, including release as age-0 fry, will be implemented for steelhead reared in similar conservation hatcheries. The behavioral characteristics of released juveniles will partly determine their fitness and interactions with extant wild fish.

The present study examines several important behavioral parameters to understand how juvenile steelhead grown in conventional and enriched environments will assimilate into post-release environments. More specifically, it investigates whether steelhead fry grown in enriched rearing environments (tanks fitted with a combination of in-water structure, underwater feeders, and overhead cover) differ from fry grown in conventional vessels, with respect to social dominance, growth, and use of woody debris structure.

Previous studies have investigated differences in aggressive behavior and social dominance relationships between populations of salmonids, or groups of juveniles, under laboratory tank conditions, and interpreted the observed differences to relationships in natural streams (Rosenau and McPhail 1987, Swain and Riddell 1990, Berejikian et al. 1996). In this study, the performance of steelhead fry in laboratory dominance trials is compared with evaluations of growth under competitive conditions in a quasi-natural stream channel. All experiments were made by removing fish from the two different rearing environments and comparing their subsequent behavior and relationships in the channels.

Methods

Study Population, Rearing Treatments, and Tagging

Eyed eggs were obtained from artificially spawned steelhead from the Skookumchuck River, Mason County, WA. This hatchery population is derived from the local wild population, and spawning protocols incorporate wild steelhead into the spawning broodstock each year. A total of 4,800 eggs were sampled from the artificial spawning of 12 males and 12 females. Eyed eggs were transported to the NMFS Big Beef Creek Research Station for incubation in constant 10° C well-water.

On 24 May, 1998 a total of 650 fish were stocked in each of six circular tanks (1.8-m diameter) filled with well-water (1,520 L in volume). Three tanks were enriched with in-water structures, the tops of two submerged Douglas fir trees. The volume taken up by the trees was approximately 315 L, or 21% of the water volume. A double layer of brown and green camouflage net hung on a circular PVC frame provided shade cover over about two-thirds of each tank. Each tank was also fitted with an underwater feed-delivery system. A PVC pipe (7.5 cm in diameter, 30 cm tall) with conical bottom was positioned above the outer rim. Well-water (13 L/min) was delivered into the pipe to create a standing column (15 cm deep) and vortex into which the food was delivered by hand. The flow carried the food to the bottom and distributed it equally through two nylon tubes (2.5 cm diameter) opening at opposite sides of the tanks near mid-water depth. More well-water (15 L/min) was delivered at the surface, making a total constant flow of 28 L/min in each tank. The remaining three tanks were conventional, and contained nothing other than a center standpipe. These tanks also received 28 L/min of well-water, all at the surface. Fish in the conventional tanks were hand-fed by scattering the food across the surface of the water.

The fish in all tanks were fed with equal frequency. At the start this was 12 times per day, gradually reduced to 4 times per day by sampling time.

Between 6 and 9 July 1998 all the fish were marked with a visible photonic tag injected into anal fin tissue, and color-coded by tank. Fish in the three enriched tanks were coded with blue, red, or white tags, and the others fish with purple, orange, or green tags. The colored tags were readily visible in tissue between several rays of the anal fin.

Experiment 1. Dominance, and aggressive behavior

(a) *Flume apparatus*

Comparisons of dominance status and aggressive behavior were conducted in two flumes (10 × 1.5 m). Each flume was divided longitudinally with a solid barrier, and laterally with screens to form 22 individual sections or cells (0.75 × 0.75 m). Not all cells were used. The substrate of each flume was a thick layer (5 cm) of gravel (1.0-1.5 cm diameter). Water depth (24 cm) was maintained by a flow of well-water (30 $L \cdot min^{-1}$) at constant temperature (12 ^{o}C), and recirculated (1,700 L/min) by submersible pumps (2 HP). Light was provided by a solid bank of wide-spectrum fluorescent lights on a simulated natural photoperiod cycle (16:8 light/dark). As one side of each flume was made of double-paned glass, all the fish in the cells could be observed. Live *Daphnia* (40 g wet weight/day) were introduced continuously by peristaltic pumps into the head box of each flume. This created a gradient down the flume, and fish in upstream cells had first access to food.

(b) *Experimental protocols*

Each dominance trial included one fish from each of the six rearing vessels. They were matched for size by placing up to five fish from a tank into one of nine small containers. Each container received fish which did not differ in weight (g) by more than 8.0%. The process was repeated for all six tanks. Within a single weight category, one fish from each tank was removed at random and then the six taken were placed simultaneously into one cell of a flume. Therefore, although the fish in the whole experiment ranged from 1.72 to 2.90 g in weight, within each replicate cell the maximum weight differences did not exceed 8.0%. In this way, any weight differences between tank representatives within a cell were randomized, and therefore non-directional with respect to treatment over the course of the experiment.

About 24 hours later, the fish in each cell were observed for three minutes to make a preliminary assessment of dominance. Dominant fish were those (a) holding a feeding station in the middle-third of the upstream half of the cell, (b) moved freely about the cell, and (c) demonstrated aggression towards others without themselves being chased or attacked. They were also uniform in body color, with prominent parr marks on a light background (Keenleyside and Yamamoto 1962, Abbott et al. 1985, Berejikian et al. 1996).

Dominant fish were then observed for seven minutes to quantify the frequency of attacks (including nips, charges, and chases), and lateral displays. Definitions of these behaviors followed Holtby et al. (1993). A fish was confirmed to be dominant only if it (a) maintained its feeding station until the end of the observation, (b) delivered more attacks than it received, (c) never exhibited submissive behavior (Keenleyside and Yamamoto 1962), and (d) never retreated when attacked or approached by other fish. The position of a dominant fish was determined again about 1.5 hours after the first observation to confirm it remained in the upstream center position.

At the end of the day each dominant fish was removed and ranked 6 (on a scale 1 through 6). The entire procedure was repeated the next day with the newly dominant fish in each cell at the end of day ranked 5. This continued until only one fish remained. Ranks were then summed for each treatment within a cell. The sums could have ranged from a maximum of 15 (the sum of ranks 6, 5 and 4) down to 6 (sum of ranks 3, 2, and 1), therefore a Wilcoxon Paired Ranks Test was used to compare the differences. A total of 24 trials were conducted between 28 July and 1 August, and 20 more trials between 4 and 8 August (n = 44).

A second analysis was made to determine if any of the six colors themselves affected dominance in a group of steelhead fry. The fry were again grouped by weight category (< 7.5%). Six fry within each category were each marked with a different color and simultaneously placed in a common cell. A total of 14 trials were carried out as before. This time the effect of color on dominance rank was analyzed by a single classification Kruskall-Wallis test (chi-square approximation).

Experiment 2. Habitat use

The experiments on habitat use were carried out in an outdoor stream channel (45 × 6 m) made of concrete. The base had a constant gradient (3%). The channel received well-water (80 L/min) which was then recirculated (5,100 L/min) by three submersible pumps (2 HP). Another pump (5 HP) extracted water (350 L/min) to recirculate through a chiller. This maintained water temperatures in the channel between 11.0-15.5° C over the course of the study, with daily fluctuations of about 2.0° C.

Sixteen replicate sections (5.0 × 3.0 m) were created in the channel by inserting a full-length longitudinal wooden divider and seven lateral weirs on which were placed wire mesh (3 mm) screens. The gravel substrate (3-5 cm diameter) was contoured with the gradient to create similar water depth and velocity profiles in all 16 sections. The average water depth (±s.d.) for all sections, profiled at intervals of 1 m along the 5 m length, was 14.8 cm (.±3.3), 19.7 cm (±2.5), 22.7 cm (±1.3), 25.6 cm (±1.4), 27.0 cm (±2.3), and 27.3 cm (±1.4) at the weir.

No artificial feed was introduced to the fish in the channel. Algal growth on the substrate and sides of the channel supported abundant aquatic insect populations. Chironomid larvae, pupae, and adults were continuously available throughout the study.

No piscine predators were introduced into the channel. Avian predators were excluded by a single layer of bird net (2.5 cm square mesh) over the entire channel.

A sliding door (13 × 61 cm) was constructed in the center of each wooden dividing wall between each pair of lateral sections. When a door was open the fry could move freely between the two. Each door could be opened or closed independently within one second by a hidden observer, and without disturbance. Once closed, the door totally confined all fish in one or the other of the paired sections (Fig. 1).

An underwater viewing chamber permitted general observations of steelhead behavior in both habitat use and growth experiments.

Two denuded Douglas fir trees (1.8 × 0.9 m diameter) were partially submerged in half the sections on either side of the channel, together with a floating log (1.2 m × 15 cm diameter) of red alder positioned on a diagonal across the upstream area. The log was held in place by lines to its respective upstream weir board and center divider (Fig. 1). These provided complex submerged structure, overhead cover, and complex flow patterns in one section of each of the eight pairs.

The first experiment was designed to test the null hypothesis that rearing treatment had no effect on habitat use (structure v. no structure) in the absence of mutual competition. Ninety fish per treatment (30 from each of 3 tanks within a treatment) were introduced into the eight paired sections on 13 July 1998, half on one side (with structure) and half on the other (without structure). The doors in the center dividers were open so fish could move freely between lateral sections. Four paired sections received fish reared in the enriched tanks, and the other four received fish reared in conventional tanks.

Seventy-two hours after the introductions all eight doors were closed, confining the fish to either structured or non-structured sections. The doors were closed at about 1100 hrs, when no external influential shadows were being cast on the channel. All fry were then removed from each section by seine net or electrofishing, and the numbers recorded and individuals identified by tag color.

On 20 July the procedure was repeated but the allocation of treatments to paired sections was reversed. The sections that received conventionally reared fish in the first set of trials received fish from the enriched tanks in the second, and vice versa. Therefore both treatments were tested in all eight adjoining sections.

A second experiment, to test the null hypothesis that rearing treatment had no effect on habitat use in the presence of mutual competition, was similar to the first with the following differences. At 1100 hrs on 27 July, 15 fish from each of the 6 rearing tanks (representing both treatments) were introduced into each of the eight paired sections. Therefore all tanks from both treatments were simultaneously represented in all eight paired sections in one set of trials. For both the competition and no-competition experiments, the percentages of fish from each treatment on the side of each pair with structures were arcsine-transformed. Comparisons between the treatments were analyzed by paired *t*-tests.

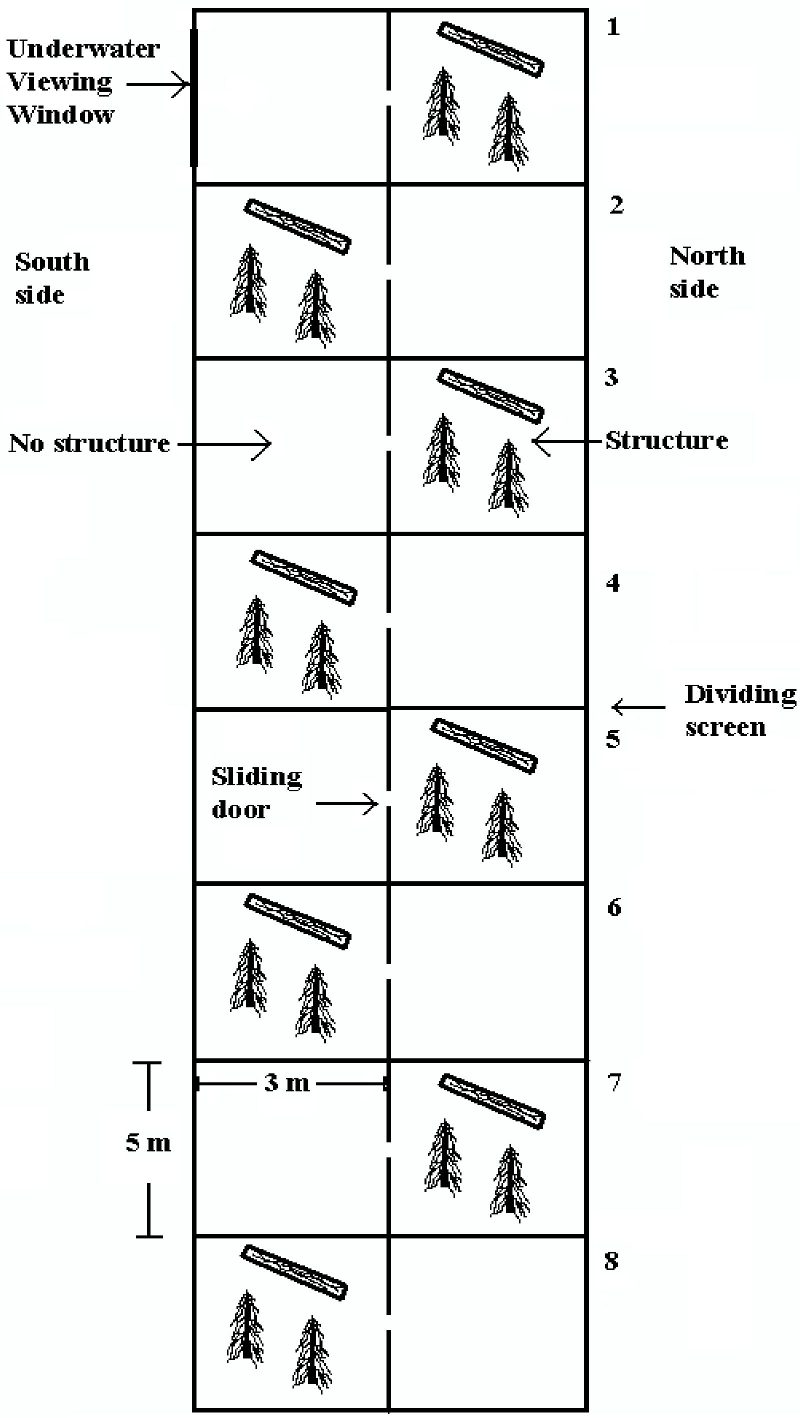

Figure 1. Top view (not to scale) of the quasi-natural stream channel used in the habitat use and growth experiments.

Experiment 3. Growth in a quasi-natural stream

Two experiments were carried out to test the null hypotheses that (a) rearing treatment (enriched v. conventional), (b) stream habitat type (structured v. non-structured), and (c) their interaction, would effect the growth rate of steelhead fry in a novel environment. These hypotheses were tested both in the presence and absence of mutual competition.

For measuring individual growth of the fry, every individual was identified with a Passive Integrative Transponder (PIT) tag, which possessed a unique alphanumeric code (Prentice et al. 1990). All fish were tagged on 10 August 1998 in the left side of the abdominal cavity. Each fish was measured (fork length, to the nearest mm), and weighed (to the nearest 0.1 g). The fish were returned to their respective rearing vessels for two more days and then transported to the stream channel at the NMFS Manchester Research Station. There was no mortality during tagging or in the two day recovery period. Fish from the enriched tanks measured 68.0 mm and weighed 3.7 g, and those from the conventional tanks measured 68.4 mm and weighed 3.5 g. There was no significant difference ($P > 0.05$) between the treatments for either parameter.

For the experiments the doors dividing lateral pairs of sections were closed, creating 16 separate sections in the stream channel. The sections on one (south) side were used to test for differences in growth under conditions of mutual competition, and those on the other (north) side were used to test for differences in growth in the absence of mutual competition (Fig. 1).

In the competition experiment, at 1200 hrs on 14 August a total of 21 fish from each treatment (7 fish per tank; 42 total) were stocked simultaneously in each section on the south side. In the non-competition experiment, at 1300 hrs the same day a total of 42 fish from each treatment (14 fish per tank) were stocked in each section on the north side, with sections 3, 4, 5, and 6 receiving fish reared in enriched tanks, and sections 1, 2, 7, and 8 receiving fish reared in conventional tanks.

All the fish in each section were removed either 20 days (competition experiment) or 21 days (non-competition experiment) later; counted, identified, measured, and weighed. The daily growth rates (%) for length were computed by the formula:

$$100*((L_t - L_0)/ L_0)t$$

where L_t is the final length (mm), L_0 is the initial length (mm), and t is the duration in days. Daily growth rates (%) for weight were computed using the same formula, but substituting weight (g) for length.

Within each section, individuals from a common rearing tank were averaged, and these means used as the experimental unit of replication for the rearing treatment effect. For the competition experiment a nested-factorial ANOVA was conducted to determine the effects of rearing treatment, habitat type, and their interaction on growth. The eight separate stream sections were nested within habitat type (four sections per habitat type; Fig. 1). For the non-competition experiment there were not enough degrees of freedom to nest stream sections within habitat type, therefore data were analyzed by a two-factor ANOVA, with rearing treatment, habitat type, and their interaction as the main effects.

Results

Experiment 1. Dominance and aggressive behavior

On each day for each of the 44 trials a dominant fish was clearly identified using the pre-established criteria. Each dominant fish defended a feeding territory in the upstream center area of its section. Its subordinates were arrayed primarily downstream. A few were occasionally positioned along the lateral margins of the cell, either level with or upstream of the dominant fish.

Fish reared in enriched vessels had a significantly higher dominance rank (mean rank = 12.1) than those reared in conventional tanks (mean rank = 8.9; Wilcoxon matched pairs test, $T = 125$, $P < 0.01$). Table 1 shows the situation each day. On day 1, dominant fish from enriched treatment ($n = 30$) and dominant fish from conventional treatments ($n = 14$) did not differ in their frequency of attacks ($T = 1.85$, 42 df, $P = 0.07$) or displays ($T = 1.25$, 42 df, $P = 0.22$) against the other five fish in the cell (Fig. 2).

The six colors used to tag the commonly-reared groups had no significant effect on dominance rank ($\chi^2 = 9.58$, 5 df, $P = 0.09$). Fry marked with orange tags had the highest mean rank (4.1), followed by purple (3.9), red (3.8), blue (3.6), green (2.9), and white (2.6).

Experiment 2. Habitat use

In the absence of mutual competition, fish from the enriched and conventional treatments demonstrated similar use of structure and no-structure habitats. After 72 hours in the stream channel, 51.6% of fish reared in enriched tanks and 50.0% of conventionally reared fish were recovered on the structure side of the paired sections ($t = 0.68$, df = 7, $P > 0.50$). When fish from both treatments were placed simultaneously into the same sections (i.e, in mutual competition), 58.9% of fish reared in the enriched tanks and 59.2% of conventionally reared fish were recovered on the structured side of the paired sections ($t = -0.11$, df = 7, $P > 0.50$).

Table 1. Results of dominance trials between steelhead fry reared in enriched and conventional tanks. Three fish from each treatment were stocked into each of 44 replicate sections (cells) of the indoor flumes. A single dominant fish on each day was removed from each section, until only two fish remained on day 5.

	Enriched			Conventional		
Day	Number of fish[1]	Dominant number (%)[2]	Empty cells[3]	Number of fish	Dominant number (%)	Empty cells
1	132	30 (68.2)	0	132	14 (31.8)	0
2	102	29 (65.9)	0	118	15 (34.1)	0
3	73	27 (61.4)	0	103	17 (38.6)	0
4	46	15 (34.1) [41.6]	8	86	29 (65.9) [58.4]	0
5	31	19 (43.2) [61.3]	13	57	25 (56.8) [38.7]	0

[1] The number of fish from each treatment present on each day of the experiment (all 44 cells combined).
[2] The percentage of dominant fish from each treatment out of 44 cells. Numbers in block parentheses [n] represent the percentage of dominant fish for only those trials in which at least one fish from both treatments remained.
[3] The number of cells in which no fish from that treatment remained, because all three fish in the cell had been removed as dominant on previous days.

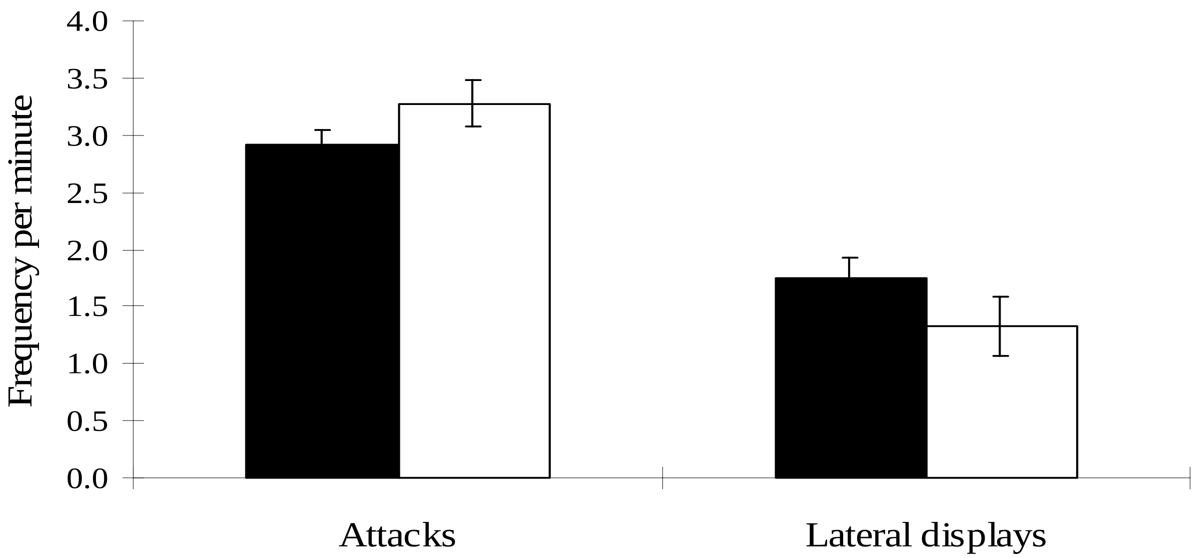

Figure 2. Frequency of aggressive attacks and lateral displays by dominant steelhead from the enriched tanks (■, n = 30) and dominant conventionally reared (, n = 14) steelhead from on day 1 of the experiment. Neither comparison was statistically significant (P > 0.05).

Experiment 3. Growth in a quasi-natural stream

In the growth experiment, conducted under conditions of mutual competition, fish reared in the enriched tanks exhibited greater increases in length ($F_{1,38} = 8.23$, $P < 0.01$) and weight ($F_{1,38} = 6.22$, $P = 0.02$) than conventionally reared fish (Fig. 2). Habitat type (i.e., the presence or absence of woody debris structure) had no effect on the rate of length ($F_{1,6} = 0.076$, $P > 0.50$) or weight ($F_{1,6} = 0.43$, $P > 0.50$) growth; there was no interaction between habitat type and rearing treatment (length: $F_{1,38} = 0.27$, $P > 0.50$; weight: $F_{1,38} = 0.96$, $P = 0.33$).

In the non-competition experiment, the two treatments did not differ in their rate of length ($F_{1,20} = 0.6$, $P = 0.45$), or weight ($F_{1,20} = 0.09$, $P > 0.50$) growth (Fig. 3). Habitat type had no significant effect on length ($F_{1,20} = 3.97$, $P = 0.06$) or weight ($F_{1,20} = 1.93$, $P = 0.18$) growth. No interaction existed between the effects of rearing treatment and habitat type for either length ($F_{1,20} = 1.99$, $P = 0.17$), or weight ($F_{1,20} = 2.06$, $P = 0.17$) growth.

Observations made from the underwater viewing chamber, situated alongside the south side of section 1 (Fig. 1) indicated that steelhead held territories close to the substrate in the swiftest currents, primarily down the center third of the channel. In agonistic contests between territory holders, the fish stationed upstream usually defeated those positioned downstream of them. Fish moved laterally from their stations to intercept food in the water column, frequently removed food from the substrate, and fed quite rarely at the surface. The most dominant territory holders exhibited a strong permanence of station, by maintaining their positions for most if not all of the 20-day experiment. They also appeared to be among the largest fish in the section by the end of the experiment. Some non-territorial fish were located in backwater currents along the side wall (window) of the section. These casual observations indicate that steelhead fry in the stream channel exhibited social patterns similar to those observed in natural streams (Edmundson et al. 1968, Everest and Chapman 1972, Berejikian 1995).

Discussion

In laboratory raceways or flumes, steelhead fry grown in conventional rearing tanks were socially dominated by size-matched competitors grown in enriched tanks. When both groups were introduced into a quasi-natural stream, fish reared in the enriched tanks grew faster than those reared in conventional tanks under conditions of mutual competition. There was no difference in the absence of competition. Therefore, in two independent experiments, steelhead grown in enriched rearing environment out-competed those grown in conventional environments.

Previous laboratory studies of juvenile salmonids found that manipulation of rearing parameters, such as density and food ration, affect competitive (agonistic) behavior measured in the rearing environments (Symons 1968, Fenderson et al. 1968),

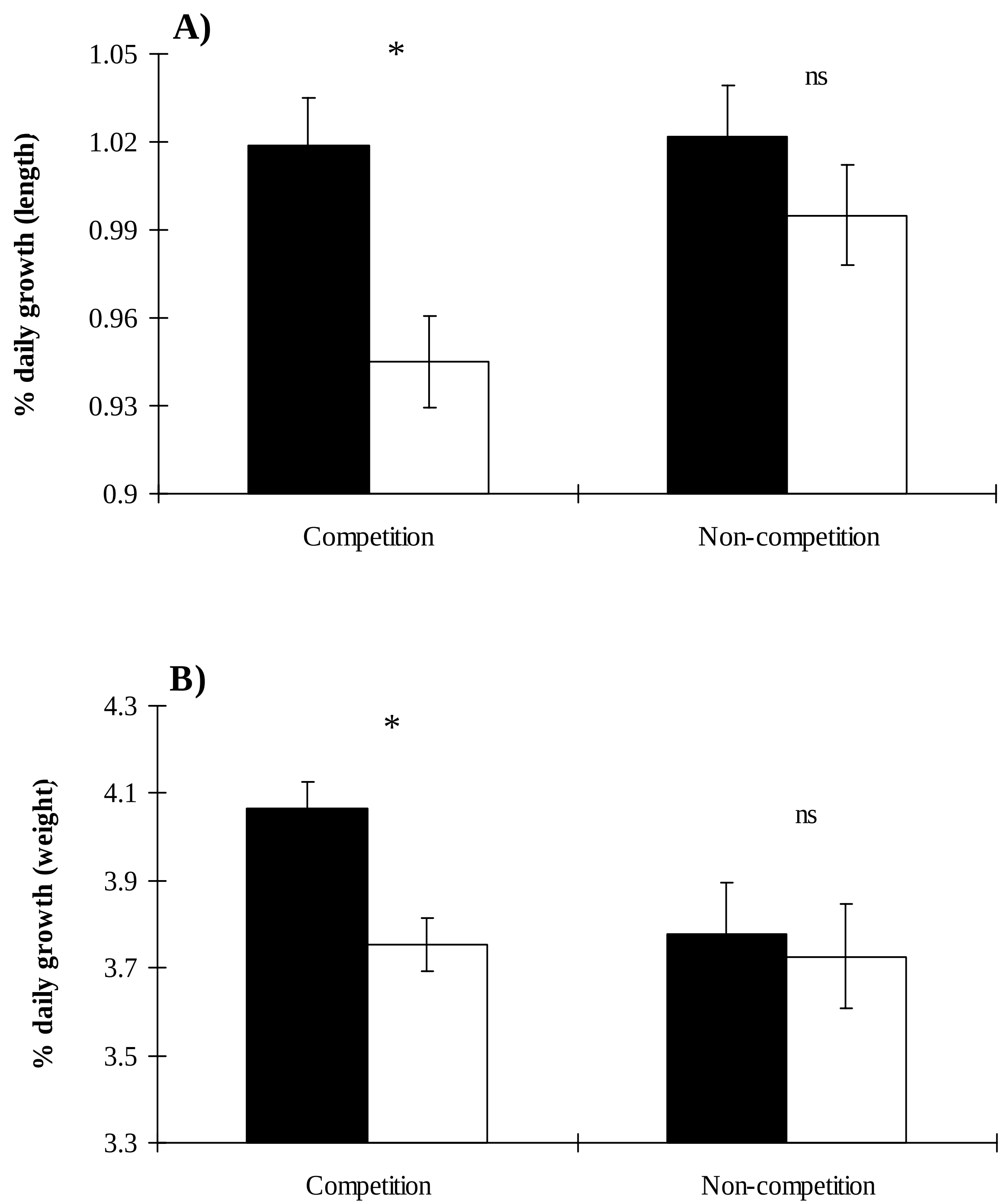

Figure 3. Differences in daily growth rates (%) in (A) length (mm) and (B) weight (g), in both competition and non-competition experiments. Bars represent rearing treatment means ($\pm$ 1 SE). Significant (*) and non-significant (ns, $P > 0.05$) results are noted above bars.

and after transfer to novel test environments (Berejikian et al. 1996). The present study demonstrates that structural modifications to hatchery rearing environments, under rearing parameters of density, food ration, and flow, affect the relative competitive ability of steelhead.

The two rearing treatments differed in several ways which could influence development of competitive behavior and performance. Existing information points towards visual isolation, defensibility of food resources, or more probably a combination of the two, as the primary causes of competitive asymmetry. Visual isolation reduces the frequency of aggressive behavior in groups or populations by reducing the frequency of encounters between potential opponents (Mesick 1988). The effect may be similar to reducing density, in that dominant fish experience reduced intruder pressure (Grant and Noakes 1987), making it possible to defend territories.

At high densities, territorial behavior of fish diminishes (Fenderson and Carpenter 1971, Wooton 1990), perhaps because the benefits associated with high levels of agonistic activity do not outweigh the costs in terms of energy expenditure (Li and Brocksen 1976) and injury (Abbott and Dill 1985). Berejikian et al. (1996) found that a hatchery population of steelhead reared at low densities exhibited greater aggressive responses to their mirror image in novel test aquariums than those reared at higher densities. Thus, low density rearing, or similarly visual isolation, may promote development of territorial and aggressive behavior by reducing intruder pressure in the rearing environment. The structure in the enriched tanks also provided reference objects used by salmonids to establish and defend territories (Hartman 1965). For territorial tactics to be adopted, however, individuals must receive an energetic ‘pay-off’ which outweighs the cost of defense.

The primary benefit of territorial behavior in the enriched rearing tanks was the presence of a defensible food source, in this case an underwater feeder. In conventional tanks food was scattered across the water surface, and could not be monopolized. Localizing food in rearing tanks increased agonistic behavior in chum salmon (Ryer and Olla 1995), but not coho (Ryer and Olla 1996). With chum there were no differences in agonistic behavior or dominance when the two food regimes were tested in novel laboratory conditions, but results of this study with steelhead show that the combination of submerged structure (which provides visual isolation) and localized underwater feed sources either improved fighting ability or motivation to defend territory, thereby increasing their competitiveness in novel stream-like environments. It is now necessary to test these two factors alone and in combination to determine their relative individual and combined developmental effects on competitive ability.

Socially dominant juvenile coho salmon exhibit higher growth rates than subordinates over time (Nielson 1992, Martel 1996). Several studies have investigated social dominance and aggressive behavior relationships of salmonids from hatchery and wild populations under laboratory conditions, and attempted to interpret the results for wild populations in natural streams (e.g., Rosenau and McPhail 1987, Swain and Riddell 1990, Berejikian et al. 1996). However, experiments on a laboratory scale can favor one group or another because of biased adaptation to an environmental factor, such as fish density (Fenderson et al. 1968). This makes inferences about laboratory-derived dominance relationships on growth or survival in natural streams less certain. For example, Huntingford and De Leaniz (1997) found the social status of Atlantic salmon in laboratory aquaria (45 L) at moderate density (55 fish/m^2) was inversely related to their subsequent growth rates in a laboratory stream channel at lower densities (3.85 fish/m^2). They speculated that, within a population of young Atlantic salmon, their competitive performance tested under different densities would depend upon their individual behavioral profiles.

In this study with steelhead, the growth performance of fish from the same treatments in a quasi-natural stream (2.8 fish/m^2) supported the results of the dominance experiment in laboratory-scale flumes (10.6 fish/m^2). Therefore the results suggest that young steelhead reared in enriched environments would out-compete those reared in conventional tanks for foraging territories in natural streams. Furthermore, the consistency of results on dominance and growth studies between the two experimental scales may give support to inferences from other laboratory-scale studies.

In the quasi-natural stream, steelhead fry from the two rearing environments exhibited similar use of woody debris structure. In natural streams age-0 steelhead use a wide range of habitats but, in general, they have been characterized as territorial, bottom-dwellers (often associated with large rubble) using shallow water habitats (Everest and Chapman 1972, Bisson et al. 1988, Bugert et al. 1991). Bugert and Bjornn (1991) found little response of age-0 steelhead to cover in a natural stream, and Hartman (1965) found them to be more prevalent in open areas than in available log cover.

In-stream woody debris provides cover habitat and velocity refuge for juvenile salmonids (Shirvell 1990). The absence of predators in these experiments provides no stimulus for young fish to learn to use the cover in enriched tanks, and consequently there was no benefit to be gained by using the structure in the stream environment. With respect to velocity refuge, midwater current velocities in the stream channel were between 0.1 and 0.36 m/s, which is within the range commonly used by steelhead fry (Bisson et al. 1988). Underwater observations of steelhead fry in the stream channel showed that territory holders generally occupied areas of swifter currents, but held positions close to the substrate. This may have provided them with sufficient velocity refuge, and the woody debris structure in the channel did not provide a greater velocity refuge.

In short, rearing tanks containing overhead cover and in-water structure did not give young steelhead a preference for similar features in a quasi-natural stream. Age-1 steelhead show much stronger preference for deeper pools and cover than age-0 steelhead (Everest and Chapman 1972, Bisson et al. 1988). Whether young steelhead reared in the two treatments would develop different affinities for cover in the stream as they grow older, or under the threat of predators (Gotceitas and Godin 1991, Reinhardt and Healey 1997) remains to be tested.

Conservation hatcheries for maintaining or restoring populations of salmonids have the option to restock hatchery-reared offspring into their ancestral habitats at various life history stages, for example, eyed egg, age-0, age-1, or smolt. A concern in these programs is the potential for domestication selection (Utter et al. 1993, Busack and Currens 1995, Reisenbichler 1996), which must be minimized to limit negative interactions of stocked fish with extant wild fish.

Selection regimes of hatchery-reared and wild salmon differ. Hatchery fish have higher survival in culture, but higher mortality after release (Waples 1991). Differences in resource acquisition, and the trade-off between competition and predator avoidance in the two environments, are theorized as possible causes of divergence in genetically-based behavioral traits of hatchery and wild populations of coho salmon (Swain and Riddell 1990) and steelhead (Berejikian et al. 1996). Clearly the reason for captive culture, the unnaturally high survival, precludes the problem of divergence from being eliminated. However, rearing fish in a more natural environment could produce hatchery fish which behave and integrate into the natural postrelease environment in ways similar to wild fish. This would reduce differences in selective pressures they experience.

Regarding ecological interactions between hatchery-reared and extant wild fish from the point of view of conservation, the concept of stocking highly competitive fish from enriched environments could be perceived as potentially detrimental to the more valuable wild fry. But if fish from enriched environments have developed more natural social behavior, then social interactions in the release environment are less likely to be disruptive to wild fish (Bachman 1984). This is possible, provided hatchery-reared fish are, (i) derived from locally adapted wild broodstock (Busack and Currens 1995, Kapuscinski 1996), (ii) restocked at densities within the carrying capacity of the target stream (Reisenbichler 1996), and (iii) within the size range of wild fish to minimize size-related effects on competition (Abbott et al. 1985, Holtby et al. 1993, Berejikian et al. 1996). Whether the protocols for enrichment used in this study produce steelhead with social behavior similar to wild fish remains to be tested, but their strong territorial behavior is consistent with such observations in nature (Edmundson et al. 1968, Everest and Chapman 1972, Berejikian 1995).

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Section 10

PROCEEDINGS OF THE NATURAL REARING SYSTEMS WORKSHOP

Sponsored by

National Marine Fisheries Service, Bonneville Power Administration, and the Washington Department of Fish and Wildlife

Edited by

Barry A. Berejikian, Anita L. LaRae, Colin R. Nash, and Thomas A. Flagg

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OPENING STATEMENT

Conference Chairman:
Conrad V.W. Mahnken
National Marine Fisheries Service, Manchester Research Station, P.O. Box 130, Manchester, WA 98353

Welcome everyone to the First Workshop on Natural Rearing Systems. First, let me thank our sponsors, the National Marine Fisheries Service, the Bonneville Power Administration, and the Washington Department of Fish and Wildlife for making this important event possible.

The salmonid hatcheries of the Northwest are being called upon to provide fish for restoration of endangered populations. But hatchery fish are distinctly different in behavior, morphology, and physiology from their wild cohorts, differences which may be related to the way they are grown. Recent studies show that altering the hatchery rearing environment to more natural conditions will produce smolts that appear, behave, and survive more like wild fish. The purpose of our Workshop is to explore a variety of topics relating to the way rearing environments can be enriched, and the skills of the young smolts can be enhanced. The proposed five topics include:

- **Habitat Enrichment**, the use of seminatural habitats,
- **Antipredator Training**, a type of pre-release skill training designed to improve survival,
- **Feeding, Growth and Release Alternatives**, or new husbandry techniques to mimic wild salmonid life-history strategies,
- **Experimental Designs** for the best scientific approach for applying these techniques, and
- A **Management** session, on the practicality of using such systems at production hatcheries.

The use of enriched environments to improve postrelease survival and reduce stereotype behavior is not unique to salmonid culture. Research on higher vertebrates has shown that simple and practical changes to the way animals are kept in captivity can have beneficial effects on their physiology and their behavior. Environmental enrichment is an increasingly popular approach for improving the well-being of captive animals and birds. Much of what we will all hear in this Workshop today and tomorrow is founded on a fairly rich literature developed by our colleagues maintaining threatened and endangered species of mammals in zoos.

Much of the past research carried out in zoos has been focused on the topic of abnormal stereotyped behavior observed in captive animals, and the relationship between this abnormal behavior and the poor health and low reproductive success of captive, warm-blooded vertebrates. Some of the most commonly observed deficiencies seen in these animals are:

- Aggressive or antisocial behavior,
- Inability to forage properly,
- Failure to develop sexually,
- Failure to initiate mating behavior,
- Poor survival of offspring,
- Greater susceptibility to disease, and
- Poor postrelease survival.

By and large zoo operators have taken three approaches to solving these problems. These are:
(i) Conserving behavior (reducing stereotyped behavior, improved foraging ability, and mating),
(ii) Reducing stress (lowering density, providing protective cover etc.), and
(iii) Improving postrelease survival (reintroduction).

Zoo managers and personnel have responded in part to these problems by creating more complex rearing habitats to provide the animals with a living experience more or less approximating to natural conditions. Specifically, enrichment techniques have been used to optimize the levels of social and physical stimulation of animals in environments which can maximize reproduction and ensure development of normal behavioral. Let me give you an example for each.

(i) Conserving behavior

Kinkajou, provided with whole fruit hanging from branches that required exploratory and manipulative behavior as an alternative to chopped fruit in a bowl, had greatly increased rates of locomotion, exploration, and foraging, and a corresponding reduction in the levels of stereotyped behavior. The one shared characteristic of this and many other zoo studies is that the animals clearly demonstrate the relationship between an enriched environment, proper foraging behavior, and the appropriate consequence of finding food. The ability to forage properly is a behavior frequently lost by animals in captivity, with serious consequences for psychological well-being.

(ii) Reducing stress

Leopard cubs, provided with enriched enclosures containing places to hide and climb, had reduced urinary cortisol levels. Coprophagy, inactivity, and excessive aggression were all reduced. Chronic stress is known to result in high levels of pituitary-adrenal activity, and can inhibit reproduction, immune response, growth, and digestion.

(iii) Improving postrelease survival

A few studies have compared Siberian ferrets, raised in an enclosed seminatural prairie dog colony, survived better after release than a second group raised in cages. Survival of the seminatural population was comparable to a translocated wild-caught population. These and other postrelease survival experiments, comparing postrelease survival of animals reared in different captive environments, suggest that creating a sufficiently enriched environment for significant gains in survival of large mammals may be difficult but accomplishable.

In conclusion let me say just this. The question frequently asked by salmonid researchers is whether the findings of colleagues working with essentially poikilothermic higher vertebrates (mammals and birds) can be applied profitably to homeothermic lower vertebrates (like reptiles, amphibians, and fishes)? Similar techniques have seldom been used by fish culturists, and few examples of altered behavior or physiology resulting from enriched aquatic environments are to be found in fish literature.

Then there is the question of innate versus learned behavior in lower and higher vertebrates. In many cases behavioral repertoires may be recovered even after many generations of absence simply by recreating the correct environmental stimuli. However, this will not be the case for behaviors learned by individuals and then passed on from generation to generation. Primates are a good example of culturally transmitted behaviors. Fishes on the other hand probably do not have a large repertoire of learned behavioral responses, relying more on innate behavior. The good news here is that innate behavioral responses are more easily recalled by providing complex environments. The bad news is that innate behavioral responses, which are genetically encoded, may be irretrievably lost through generations of inbreeding.

The focus of most of the work carried out at zoos has been on culture in captivity followed by the reintroduction to the wild of endangered predatory mammals and large herbivores. But postrelease studies of survival of these rare species have been difficult to carry out because the scarcity of these animals limits any experimental design. The result is that little productive research on the fate of released zoo animals has been carried out. Those of us working to improve postrelease survival of salmonids are less constrained by the numbers of test organisms but more by hatchery fish survival rates. Nevertheless, we have opportunities unequalled by our colleagues working with zoo animals. We have the ability to work with very large numbers of test animals, to replicate experiments, to control growth and development, and finally to choose among a variety of available test sites for conduct our studies.

Finally, many of us believe that culturing salmonids in seminatural raceway habitats may also modify their behavior, physiology, and morphology, and improve their postrelease survival. There is now limited evidence that seminatural rearing can improve postrelease survival. However, the biological mechanisms are still largely unknown. One of the primary purposes of this Workshop is to present the results of recent years of experimentation that examines the effect of culturing salmonids in enriched environments, including overhead cover, in-stream structures, different substrates, and what are believed to be non-intrusive feed delivery systems.

Again, let me thank you all for coming, and particularly our colleagues presenting the 27 individual project papers we will hear in these next two days. Also let me thank those who have agreed to moderate the Sessions and to sit on the expert panels. In addition to a brief period for questions and answers after each presentation, each of the five Sessions will end with open discussion. Again all the presenters will be available to answer more questions, and I encourage you all to participate.

SESSION I. HABITAT ENRICHMENT

Session Moderators:
Thomas A. Flagg[1] and Jeff Gislason[2]
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<u>Presentation 1.1:</u>

Semi-natural Raceway Habitats: A Tool for Increasing Postrelease Survival of Chinook Salmon

Desmond J. Maynard[1], Eugene P. Tezak[1], Michael Crewson[1], Deborah A. Frost[1], Thomas A. Flagg[1], Steve L. Schroder[2], Chuck Johnson[2], and Conrad V.W. Mahnken[1]
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Synopsis

There is growing concern that hatchery-reared salmonids die at higher rates than their wild-reared counterparts. Seminatural raceway habitats (raceways fitted with overhead cover, in-stream structure, and substrate) may improve postrelease survival of hatchery-reared salmonids by better acclimating fish to their postrelease environment. Four studies were carried out to determine if seminatural raceway habitats increased postrelease survival of chinook salmon.

In the first study, ocean type chinook salmon were reared for 4 months from swim-up to smoltification in raceways (400 L) with opaque overhead cover, plastic aquarium plant structure, and sand or gravel substrates. During culture, the seminaturally-reared fish exhibited escalated agonistic behavior, compared with conventionally-reared fish. At the end of rearing, the skin hue and chroma of seminaturally-reared fish differed significantly ($P < 0.05$) from conventionally-reared fish. This effectively enhanced their ability to camouflage to their postrelease stream background. When released into a small coastal stream (Little Anderson Creek on Hood Canal) the survival to a collection weir 2.2 km downstream was significantly ($P < 0.05$) higher (51%) for seminaturally-reared than conventionally-reared fish.

In the second study, an acclimation approach to seminatural raceway habitat- rearing was evaluated. Stream-type chinook salmon were initially reared in barren circular tanks for 9 months after swim-up. These fish were then transferred to either seminatural or conventional raceways (400 L) for the final 4 months of experimental rearing. The seminatural habitat in this experiment consisted of opaque overhead covers, ornamental junipers for structure, and gravel substrate. When released as smolts into the Yakima River under clear water conditions, the post-release survival of seminaturally-reared fish to a collection weir 225 km downstream was significantly ($P < 0.05$) and higher (24%) than conventionally-reared fish. When released under turbid water conditions, there was no significant difference in the postrelease survival of the two treatment groups.

In the third study, culture vessel size was increased (5947 L), and ocean-type chinook salmon were reared for about 3 months (from swim-up to smoltification) in raceways fitted with camouflage net covers, fir tree structure, gravel substrate, and an underwater feed-delivery system. At the end of rearing, the skin coloration of seminaturally-reared fish again appeared to be better suited for blending into the natural stream background. When released into a tributary of the Satsop River (Bingham Creek), the seminaturally-reared fish averaged significantly ($P < 0.05$) higher (26%) postrelease survival to a collection weir 21 km downstream than their conventionally-reared counterparts.

In the last study, culture vessel size was increased further, and ocean type chinook salmon were reared for more than 3 months (from swim-up to smoltification) in raceways outfitted with camouflage net covers, fir tree structure, and a gravel substrate. When released into Forks Creek, a tributary of the Willapa River, the seminaturally-reared and conventionally-reared fish averaged similar and non-significantly different ($P > 0.05$) in-stream postrelease survival. The dark coloration of the control vessels and high turbidity driven sedimentation in the control raceways may have resulted in both rearing types developing similar camouflage coloration.

Where it was analyzed, the skin of seminaturally-reared fish develop a more natural camouflage coloration than conventionally-reared fish. This suggests the higher postrelease survival of seminaturally-reared fish results from their lower predator vulnerability, due to their enhanced crypsis in the stream environment. Seminatural raceway habitats provide fish culturists a tool to increase the postrelease survival of salmon released in fisheries enhancement and conservation programs.

Workshop Discussion

Q. How long were the fish reared in these containers prior to release?

A. In most cases, four months; in others, three months.

Q. I would observe that the sides of most of the production raceways we use at our hatcheries are algae-covered. I think the gray background you depicted in your NATURES experiment is not necessarily true of a lot of production hatcheries. The other thing I would point out is that many of production raceways currently in use are old, and have a lot of exposed aggregate on the bottom, thus mimicking your pea gravel. In addition, much of the production in this basin is reared in large ponds, which have a lot of sediment on the bottom.

A. In response to your first point, most of the newer production facilities do not have algae growth in the raceways. They tend to have light gray concrete walls. When you go to the older facilities, I agree with you; there is a lot of algae growth and exposed aggregate. Unfortunately, as we try to update our facilities a lot of those older raceways are being replaced with bright new concrete. As for the sediment in the larger rearing ponds this may be an advantage; however, there are a number of facilities that have replaced their dirt-bottomed ponds with shiny new concrete ponds. My point is that, in the process of making those renovations, you may want to give some thought to the rearing environment so that it looks a little more like a natural stream.

Q. In the Forks Creek experiment, at what kind of densities were the fish reared? Also, how long after the fish released did you begin to see recoveries at the juvenile collection facilities?

A. In response to your first question, our finishing densities were pushing one pound per cubic foot. For the next question, at Forks Creek, we got a record high fish recovery of 70%. Most of those fish reached the collection facility within two days for a distance of 17 - 21 km.

<u>Presentation 1.2:</u>

Evaluating the Effects of Complex Rearing Habitats on Juvenile Fall Chinook

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Synopsis

Previous studies have showed that the postrelease survival of cultured fall chinook can be improved when fish are reared in raceways containing gravel substrates, intra-gravel filtration, underwater feeders, in-water structure, and overhead cover. Our objectives were to determine whether similar gains could be achieved by using only a few of these components or by reducing the length of time the fish were cultured in such environments. In 1996 we evaluated the effects of 7 different rearing treatments on the in-culture performance and postrelease survival of fall chinook. In the control or OCT (Optimal Conventional Treatment) case the fish were fed by hand from the surface and nothing was added to the 2 m circular tanks we used as rearing vessels. The remaining 6 treatments all had 90% of their surface area covered with camouflage netting. In one treatment this was the only added feature. An additional single element, either an underwater feeder, a filter that was covered with a scattered layer of pea gravel or two submerged panels of camouflage netting were added to create three additional rearing regimes. The remaining two treatments possessed covers, underwater feeders, intra gravel filters, and in-water structure, however fish were reared under these conditions for varying periods of time.

Those experiencing a full NATS (Natural Rearing Systems) treatment were held throughout their entire 92 - 105 day rearing period. Fish exposed to the LNAT (Limited) treatment, on the other hand, were reared under OCT conditions and then exposed to a NAT regime over the last 30 days of their rearing period. None of the rearing treatments appeared to affect the growth of the fish. Differences, however, did occur in their in-culture survival. Fish reared in tanks without substrates or in- water structure had mortality rates that were less than 1%, fish held in tanks with in-water structure experienced slightly higher mortality (1.5%) while the NAT, LNAT, and cover-substrate tanks had the highest mortality rates (up to 20%). The rearing environments also affected the color patterns of the fish. Individuals from the NAT and LNAT tanks had pronounced parr marks, colored fins, and heavy melanic spotting and thus appeared to be more cryptic than fish produced from the other rearing treatments.

The postrelease survival of fish originating from each treatment was evaluated by making two separate releases into Bingham Creek and allowing the fish to migrate 21 km before being recaptured. In the first, fish reared under the NATS regime had a 16% higher survival rate than those held in the OCT tanks, no other survival differences were seen. In the second, fish originating from the LNAT tanks had significantly lower survival rates than those originating from the other rearing treatments. Just prior to being released, these fish had experienced a *Costia* outbreak and we speculate that this may have adversely affected their survival.

In 1997 we examined the effects of 4 different substrates and a NATS treatment on the in-culture performance and postrelease survival of fall chinook at Bingham Creek. As in 1996, the fish were liberated into Bingham Creek after a 3 month rearing period. The in-stream survival rates of these groups have not yet been evaluated. Distinct differences in the color patterns of the fish were noted and mortality was slightly higher than 2% across all the groups. We did see slightly more fin and opercle erosion on fish reared in the NATS tanks however.

Workshop Discussion

Q. For the group you released on 20 June you had 46% survival, but for the group you released on 3 July 3 you saw only 24% survival. Was there a big difference in temperature between those two release dates?

A. There was undoubtedly a temperature effect. We have been talking about doing a stepwise regression analysis using a whole suite of variables, such as treatment, water temperature, mortality prior to release, etc., to figure out which factors are predominantly responsible for mortality.

Q. Did you see any difference in condition factors between treatments?

A. We did take lengths and weights on these fish, and have looked to some extent at condition factors, but the basic answer to your question is, No.

<u>Presentation 1.3:</u>

A New Hatchery Concept: A Lesson from Sockeye Spawning Channels

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Synopsis

In general, and with notable exceptions, salmon production management through the use of hatcheries has not been successful. Much of the problem can be traced directly to the disregard of specific stock requirements, or the synchrony that has evolved in a population with its environment. Translocation, release strategies, and breeding programs all have contributed to hatchery failure.

However, another major problem is the environment that our hatcheries represent. Density, conditioning, and the totally unnatural basin habitat have prepared the releases with the wrong expectations. The approach to correct this serious dilemma is to re-establish natural rearing conditions and to follow the stock concept in all enhancement and supplementation programs. That means we need to revise hatcheries, which I am referring to as the “New Hatchery Concept”. In this approach, two critical priorities are required. (1) The first is to develop spawning and rearing facilities that represent natural salmonid habitat. These facilities will not be concrete basins in which we artificially feed juvenile salmonids at densities of 15 lb/gpm of flow, but rather as constructed natural stream channels with pool and riffle environments that promote the production of natural feed, and stocked at rearing densities appropriate for the carrying capacity of thc stream. (2) The second is that only the native stocks of salmon or steelhead located in the reach of the river being enhanced will be used.

The best model for such a concept is the sockeye spawning channels on the Fraser River. Constructed streams for introduction rearing in natural-type habitat can be developed where such habitat has been lost for stream dwelling species. River water diverted to prepared side channels and basins developed for that purpose can supplement present habitat or replace habitat lost through river development. Such habitat development can also take place in the upper sections of irrigation diversions, and would provide opportunities to involve other water users in salmonid rehabilitation.

Workshop Discussion
No discussion was recorded

Presentation 1.4:

The Methow Salmon Hatchery "Supplementation Facility"

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Synopsis
As part of the Methow River Spring Chinook Enhancement Project, the Methow Salmon Hatchery was built for the sole purpose of enhancing the natural production of spring chinook salmon in each of three sub-basins in the Methow River, without affecting the genetic characteristics of each stock. The project also included the construction of remote satellite acclimation ponds and adult trapping facilities. The hatchery makes use of innovative design and operations features, which closely resemble conditioning found in nature. The hatchery and associated facilities were designed by Fish Pro, Inc., built with funding from Douglas Co. PUD, and operated by the Washington Department of Fish and Wildlife.

Introduction

Location/description. The main production hatchery is located in north central Washington near Winthrop, along the Methow River. Facilities consist of a hatchery building with 300 iso-buckets, 45 vertical incubators and 24 – 3' × 15' × 2.5' fiberglass starter tanks. Outside, there are 12-8' × 80' × 4' covered concrete raceways, 3 0' × 00' × 4' covered concrete adult ponds, 1 – 110' × 40' × 4.5' on-site hypalon-lined rearing pond, 2 – 110' × 40' × 4.5' off-site gravel-lined acclimation ponds, 3 off-site adult collection facilities, a surface water intake, and ground water supply. The hatchery program calls for a production goal of 750,000 spring chinook smolts @ 15/lb divided equally between the Twisp, Chewuch, and Methow Rivers.

Water supply/intake. Surface water is supplied to the hatchery by an intake that virtually takes care of itself. The five main intake screens are set at a 45 degree angle to the flow of water allowing for sweeping action across the screens, leaves, and other floating debris are easily flushed past the screens to a partially open gate. An overflow weir upstream of the intake, within the structure, sets the minimum head needed to operate all production facilities by gravity flow. In addition, there is an ice prevention system which utilizes a small air compressor to charge diffusion piping located on a support rack under the intake screens. At low temperatures the system produces fine bubbles of air that rise past the intake screens helping in the prevention of icing. Similar intakes are also in operation supplying the Chewuch and Twisp acclimation ponds. Surface water temperatures range from 32-65 degrees Fahrenheit.

Adult Collection and Molding

Capture and transport. There are adult traps located upstream at each of the three rivers (Twisp, Chewuch, and Methow). Barriers to fish passage at each site help to divert returning adults into collection areas for subsequent transport back to the main hatchery. A floating barrier is utilized on the Twisp, a modified/remodeled denile-type fishway on the Chewuch, and a vertical slot fishway, with trapping bypass on the Methow. Once in the collection area, the adults are marked and placed into an innertube to be carried up to a 300 gallon tank used in transport. Adults which are not needed for the hatchery program can also be easily passed upstream through a small opening in the trap area.

Adult ponds. After a short hauling time, the adults are placed into one of three adult ponds by allowing them to travel down a PVC tube from the tank into the pond. Having three ponds allows us to keep each of the three stocks separate throughout the holding process. Adult ponds are 9' × 90' × 4' concrete with both a surface water and well water supply. A spray system along the sides helps to keep the adults calm during the holding period. Barriers along the sides and at both ends of the pond keep adults from jumping out. A perimeter fence with ground pressure sensors and motion lighting help to add security to the system. An epoxy paint seals the walls of the ponds and eliminates any abrasions on the adults.

Spawning and Incubation

Spawning area. There is an individual spawning area at the head end of each adult pond. This allows greater ease in disinfection between ponds on spawning days. Two separate drains from the spawning area allow spawning wastes to be diverted into the septic system if needed. Spawning protocol calls for 1:1 spawning of all fish with disinfection between individual females. Iso-buckets are used for mixing eggs with milt prior to water hardening.

Incubation rooms. There are three separate incubation rooms with the ability to isolate-incubate up to 100 females per room. Iso-buckets are used until viral and ELIZA test results are known. Eyed eggs are then transferred to 15 vertical incubators and arranged based on those test results. An automated formalin delivery system can treat the iso-buckets as well as the vertical incubators through a system of stainless steel piping and valves. The formalin mixing and delivery point is located in a separate specially designed room in the hatchery. Each incubation room is positioned so that fry transfer to the start tank room easily.

Rearing

Early rearing/start tanks. From the vertical incubators fry are transferred to starter tanks. The tanks are molded fiberglass, dark blue in color, and sized 3' × 15' × 2.5'. With 24 of these tanks the ability to separate different egg lots based on fry size and/or propensity for disease problems is greatly enhanced. Loadings are kept at or below .125 lb/cu ft/in at all times. Overhead lighting is adjustable to simulate natural conditions during the first feeding. Twin drain sumps can divert waste water to the clarifier during cleaning as can an inline vacuum system. CWT marking takes place in these tanks just prior to transfer to outside raceways.

Covered raceways. Twelve covered raceways are located away from active areas and available for rearing until yearling transfer to acclimation ponds. Each pond is 8' × 80' × 4' with a well water and surface water supply. Brown-pigmented concrete creates a more subdued environment which the fish respond well to. PVC vinyl covers also add to the natural lighting to shade the ponds from the bright sunlight. Baffles in the ponds create currents which help to clean the bottom as well as giving the fish different velocities to choose from. Water temperatures can be tempered to closely match the river cycle. After a year the fish are then transferred to their respective acclimation pond for release.

Acclimation

Release ponds. There are three acclimation ponds; 1 on the Twisp River, 1 on the Chewuch River, and 1 on the Methow River. The ponds are all 40' × 110' × 4' with predator fencing around all sides and low level overhead bird protection. Yearling spring chinook are transferred in March from the main hatchery and allowed to leave the pond volitionally after 6 weeks of acclimation tie. Size and time of release are 15/lb and April 15th respectively. The acclimation ponds incorporate a variety of features which help to mimic conditions found in the natural environment.

Natural rearing additions. All acclimation ponds have a camouflage-type netting over certain areas. The shading that is produced allows the fish to escape the bright sunlight and to experience different environments. The netting is easy to feed through and is tough enough to withstand severe temperature changes. The vegetation around and inside of the ponds has been allowed to grow up contributing to an increase in insect production. The weed growth also creates areas for fish to rest and/or hide. Gravel substrate along the sides and bottom of each pond also help to acclimate the fish to conditions which they will find upon final release into the river. River water is used to further acclimate the fish to temperature changes and to increase homing success of adults.

Returns

Since the hatchery's first brood year was 1992, adult returns are only just begging to show up in the Methow system. We did see some 4-year-old fish this year from both the Chewuch and Twisp releases in 1993. Next year's 5-year-old returns should give us some additional data with which to make some observations on the overall program.

Summary

The goal of the Methow Salmon Hatchery is to increase the number of returning adults to the Methow System without sacrificing a loss in the genetic integrity of the three target stocks. Isolation techniques, small manageable starter tanks, acclimation facilities, stringent spawning guidelines, and natural rearing will only add to our ability to achieve that goal.

Workshop Discussion

Q. What was the flow rate in the baffled ponds?

A. We put 1,000 gpm through the pond.

Q. How long were the fish kept in the acclimation pond?

A. Between four and six weeks. In March it is still winter up there, so there is no way to introduce the fish to the acclimation ponds any earlier.

Presentation 1.5:

Evaluation of a Semi-Natural Rearing Pond for Coho Salmon

Howard J. Fuss and James Byrne

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Synopsis

Conventional fish rearing practices are thought to reduce the survival of hatchery fish relative to wild fish due to relaxation of selection pressures and feeding of sub-optimal diets. Some recent data suggests some key differences in physiology between hatchery and wild fish at the time of seaward migration. For example, hatchery fish had reduced tolerance to seawater, and had significant declines in hematocrit, significant increases in circulating plasma cortisol concentration, fewer chloride cells and lower specific activities of the enzymes Na + K+ ATPase and citrate synthase. Chinook salmon reared in NATURES tanks had more natural type body camouflage coloration patterns, a greater fright response to overhead movement, and a 25-50% survival advantage during migration in the stream corridor when released in clear running streams over conventionally reared groups. Although NATURES technology can be retrofitted to existing hatchery raceway systems, it has not been tested under full production situations, nor has it been demonstrated with species other than chinook. Increases in survival attributable to NATURES rearing would have to offset the loss in numbers released to be of benefit in production oriented hatcheries.

If NATURES rearing can be done at production levels and impart a survival advantage, then increased production of adults over conventional rearing would occur. However, if NATURES rearing requires reductions in the number of fish released to be successful, the fish reared in these converted vessels would have to survive at much higher rates than conventionally reared fish. The purpose of this study is to determine if a conventional rearing pond can be converted to a seminatural rearing pond and successfully produce coho smolts. This study incorporates a rearing scenario that could be realistically incorporated in most hatchery operations. The study addresses two questions: (1) Do seminatural rearing conditions at a hatchery produce smolts that survive at higher levels, and have different smolt characteristics than those reared under standard conditions? (2) If increased survival does occur, is it high enough to offset losses in production that would occur using the "seminatural" rearing environment?

We addressed these questions by modifying a single dirt bottomed rearing pond at the Elochoman Salmon Hatchery to simulate a natural off-channel rearing pond by adding pit run gravel and large woody debris (LWD) to the pond. Thirty thousand coho fry, initially reared in conventional concrete raceways, were added to the treatment pond in June 1996. Coho, from the same source as the treatment pond, were stocked into a dirt bottomed rearing pond (control, pond 21) in early February at about 9g. A total of 263,000 fish were stocked in the pond. Fish in the treatment pond were allowed to feed on natural feed throughout the rearing period but were supplemented with commercial feed using Babington Response Feeders. Fish in the control pond were fed a daily ration by hand. Growth rates in each pond were programmed to achieve a release size of 17 fpp. Electronic fish counters were placed at the outlet of each rearing pond to count daily outmigration. Screens were lifted and fish allowed to emigrate volitionally beginning on April 14, 1997. Fish were allowed to emigrate until each pond was nearly empty. A minimum of 15 smolts from each pond were captured each week after passing through the fish counter, sacrificed, and gill epithelial tissue was removed and handled as per methods described by Schrock et al. (1994). Likewise, blood was collected from the same fish and analyzed for plasma chloride and plasma cortisol levels. Coded wire tags were applied to all 30,000 fish in the treatment pond and 30,000 (BPA Index) of the control pond to calculate overall survival. Results presented will be from the first of a three year study.

Workshop Discussion

Q. What was your survival rate? What percentage of the fish you put in survived?
A. It was well over 95%.

<u>Presentation 1.6:</u>

Addition of Floating and Bottom Structure to Hatchery Raceways

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Synopsis

Many hatcheries operated by WDFW are designed to mass-produce fish in unnatural conditions. While redesigning these facilities to more closely resemble a natural stream may be impractical, some simple and immediate modifications are possible. If the use of inexpensive and convenient in-pond structures produce fish that are better adapted to the natural environment and increase survival, they could be widely adopted. We are testing the hypothesis that the addition of floating pond covers or artificial bottom structures will increase the survival of hatchery reared coho salmon.

In three consecutive years, we are rearing coho at Solduc Hatchery with three different treatments during the final two months before release. Two ponds are control ponds to which no structures are added. Floating pond covers made from polypipe and camouflage netting are secured in two ponds, and bottom structures made from plastic fish totes are added to two ponds. All ponds contain the same number of fish and all are fed with demand feeders. To monitor survival at return, 25,000 fish in each pond receive a unique coded-wire tag. During rearing, growth and behavior is compared between the ponds.

The study began in 1997. Because of a disease outbreak, we were only able to use four ponds for the study, so we left out the treatments with the bottom structures. The floating structures were put into the ponds in mid-February, 8 weeks before release. The fish quickly began using the structures, which were covered with algae shortly after putting them in the ponds. The algae probably increased their efficiency, and were not a concern for fish health. No differences in growth were observed between the control fish and the treated fish, but at release, the fish from the treatment pond were more smolted than those in the control ponds. This project will continue for two more years, and the first tags will be recovered in 1999.

Workshop Discussion
No discussions were recorded

Presentation 1.7:

Physiological Effects and Behavioral Characteristics of Hatchery-Reared Coho Salmon Juveniles Under Riparian-Like Covers

Stanley D. Smith[1], Alec G. Maule[1], Robin M. Schrock[1], Phillip V. Haner[1], Jack D. Hotchkiss[1], and James J. Rockowski[2]

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Synopsis

In light of regional concerns regarding the effects that hatchery populations of Pacific salmon may have on the overall survival of wild stocks, we assessed the physiological and behavioral effects of riparian-like covers on hatchery reared coho salmon over a two-year period to determine if more wild-like behavior could be "taught" to hatchery stocks. Two control (uncovered) and two treatment (covered) raceways were used for the study at the USFWS Willard National Fish Hatchery. To assess physiological effects we sampled approximately monthly for length and weight, plasma cortisol, gill Na+-K+-ATPase, plasma and mucus lysozyme. In year two we also monitored skin reflectance as a measure of smolt development. At the end of year one at the time or release, we conducted acute stress and seawater challenge tests to further evaluate physiological variations between control and treatment groups. With few exceptions, we found no significant differences in any of the physiological indices during the overall study. However, we did observe that mucus lysozyme levels correlate very well with

plasma cortisol levels which may lead to the development of a new non-lethal sampling technique for smoltification or stress. We also observed behavioral differences between the groups, such as better use of the entire raceway by the treatment group that may warrant further studies to determine if stressors are relieved by reducing overcrowding

Workshop Discussion

Q. Did you have any problems with fish jumping through those covers?

A. No.

Presentation 1.8:

The Yakima/Klickitat Fisheries Project

David Fast

Yakima Indian Nation, P.O. Box 151, Toppenish, WA 98948

Synopsis

The Yakima/Klickitat Fisheries Project (YEP) was first approved by the Northwest Power Planning Council (NPPC) in 1982 as a cluster of production facilities in both the Yakima and Klickitat River Basins to enhance the fishery for the Yakima Indian Nation and other harvesters. The project has been modified to include research activities testing the assumption that new supplementation methods could increase natural production and improve harvest opportunities while maintaining the long-term genetic fitness of the wild and native salmonid populations and keeping the adverse ecological interactions within acceptable limits. The Upper Yakima Spring Chinook Supplementation Complex is the first step in that process. The facilities designed and constructed to enable these production and research activities include the broodstock collection facilities at Roza dam, the juvenile and adult monitoring stations at Roza and Chandler dams, and the Upper Yakima Supplementation Complex at Cle Elum. Three acclimation sites in the upper Yakima will complete this first phase of the project.

Spring Chinook adults have been randomly selected from the collection facility at Roza and transported to the adult holding pond at the Cle Elum complex. These salmon will be spawned in September and the eggs will be incubated through the winter. The eggs will be divided into two equal groups, the Semi-Natural Treatment (SNT) and the Optimum Conventional Treatment (OCT). The juveniles will be reared for one year under these treatments, and transported to the acclimation facilities in the winter of l999. The acclimation ponds are designed to allow volitional release into the adjacent streams. Each treatment and control group will be marked for visual identification at the smolt collection facilities, and as returning adults. It is expected that the survival to smolt at Chandler will allow an early evaluation of the treatment vs. the control groups, but final conclusions will be based on the number of adults returning to their release area and their success in reproducing in the natural environment

Workshop Discussion

Q. What water source are you using in the hatchery, and do you use it for all life-stages?

A. We will be using a combination of pumped river water and well water. The well water will be used to thermally temper the river water, to keep it in the optimal temperature range and mimic the temperature regime the wild fish would be experiencing.

Q. How large will the fish be at release?

A. Fifteen to the pound.

Q. One comment. I realize that you are categorizing fish based on the old criteria, but a September 1 spawner, to me, is really a summer chinook, not a spring chinook. The point is, summer chinook are not extinct in the Yakima system, and are probably well-represented, if you look at spawn timing vs. other criteria. The other point I want to make is that, while the care you are taking of the genetics of the stock in the hatchery is commendable, I have some real concerns about your interception of fish at Rosa. You do not know where those fish are going to go if they're left to spawn naturally, but now you are grouping them, and will subsequently distribute them. If you pursue this course of action, substock evolution will be completely eliminated. Personally, I think you would be much better off to collect spawners from the spawning grounds, or even at the hatchery, which is further up the system -- I think it is a mistake to assume that the fish arriving at Rosa are all the same.

A. Based on electrophoretic analysis, we have made the assumption that these fish are all the same stock. It all depends on what level you want to take it to. It could be argued that every different riffle represents a different stock, because fish home back to the same riffle generation after generation. My concern is that, 20 or 30 years from now, you're going to end up with a single, homogenized population, rather than one that is made up of fish homing to stock-specific areas.

Q. Comment. If that comes to pass, your genetic fitness is going to be lower than it would otherwise have been.

Presentation 1.9:

The Yakima Fisheries Project Spring Chinook Supplementation Program: An Experimental Platform for Large-Scale Tests of Naturalized Rearing Regimes

Craig A. Busack

Monitoring Implementation Planning Team, Yakima Fisheries Project,
Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, WA 98501-1091

Synopsis

The purpose of the Yakima Fisheries Project Spring Chinook Supplementation Program, a joint effort by the Yakima Indian Nation (YIN) and the Washington Department of Fish and Wildlife (funded by the Bonneville Power Administration), is to test whether hatchery supplementation can be used to increase natural production and harvest while keeping genetic and ecological impacts within acceptable levels. Since supplementation cannot possibly increase natural production without adequate adult returns, the postrelease survival of hatchery smolts is a major concern in this program. Accordingly, a key component of the program is evaluation of naturalized rearing regimes. Half the programmed 810,000 smolts will be reared and acclimated

under a naturalized regime termed the seminatural treatment (SNT), and the other half under a high-quality regime lacking naturalized rearing features, the optimal conventional treatment (OCT). Naturalized features of the SNT will include cover, underwater feeding, structure, substrate, and probably antipredator conditioning. The treatments will begin at ponding when the fish will be placed into 18 identical (except for SNT modifications) raceways at the YSP's Cle Elum hatchery on the Upper Yakima River and be continued in 18 raceways (six per site) at three replicate acclimation sites at Easton, Thorp, and on the North Fork of the Teanaway River. This physical layout was designed to allow the detection of a 50% difference in survival to adulthood, with 90% power, between SNT and OCT from a single year's release. Monitoring and tagging facilities at Roza and Prosser dams will allow close monitoring not only of survival, but also of morphological and behavioral differences between the two treatments as fish migrate downstream. The program began collecting broodstock this year, so data collection from outmigrating smolts and from returning adults will begin in 1998 and 2001, respectively.

Workshop Discussion

Q. I am curious why your experimental design and your power analysis are focused on single years?

A. Typically, in these kinds of studies, we are interested in making inferences that extend a fair distance into the future. We want to know whether we will see a consistent survival advantage across a number of years and a wide range of environmental conditions.

Q. Why would you design a study where your analysis was based on a single year only?

A. We wanted it to be powerful enough to tell us as much as possible about each year's release. We do not intend to run this study for a single year, then stop it. We will certainly look at how things vary from year to year.

Q. What about disease problems, and how those might potentially impact your experimental releases?

A. We will just have to see how big a problem it is. If it turns out to be a big problem, then we will have to make some decisions.

Q. What consideration have you given to life history differences in your broodstock?

A. We have not done a spawning ground area-by-spawning ground area comparison of life history differences. We have sampled the fish electrophoretically, and find no differences. In my opinion, it is simply impossible to run a broodstock program in such a way that no geneticist will quibble with your methodology. In this case, we are assuming that all of the fish arriving at Rosa Dam are genetically homogeneous and, as that has been pointed out, may be a naive assumption. But there are problems associated with tributary-specific broodstock collection as well. The bottom line, for this program, is that we have tried as hard as we can to collect broodstock randomly.

Presentation 1.10:

Natural Rearing May Not Produce Wild-Type Fish When Genetic Changes in Behavior Have Occurred in a Hatchery Population

Gayle Brown, Steve Rubin and Jay Hensleigh
U.S. Geological Survey, Biological Resources Division, Northwest Biological Science Center-Seattle, 6505 NE 65th St., Seattle, WA 98115

Synopsis

Substantial evidence exists to show that unintended genetic changes in behavior and other characteristics have occurred in various hatchery programs with a variety of species. We present new evidence of genetic change in another behavioral characteristic, willingness to feed at the surface, in progeny of a 5-generation hatchery population of steelhead compared to progeny of wild steelhead from a tributary close to the hatchery.

Groups of siblings of both lineages were collected as gametes and reared simultaneously under similar conditions in four different environments: a production hatchery, our laboratory, artificial streams and a natural stream. In laboratory tests of fish reared in the first three environments, hatchery-lineage juveniles always fed significantly more willingly at the surface regardless of rearing environment. The results from hybrids were intermediate. Snorkelling observations of fish after one year of rearing in the natural stream also showed differences in frequency of surface feeding as well as positioning in the water column and distance from cover.

Our conclusion from these results is that the behavioral differences between the hatchery and wild populations are genetically based and were likely have been produced by natural or other inadvertent selection acting to increase fitness for rearing in the hatchery environment. Our results also suggest that the hatchery population has diverged enough that the combination of extended natural experience and natural mortality were insufficient to make the behavior of hatchery-lineage juveniles indistinguishable from wild-lineage juveniles. Supplementation programs or other conservation-oriented hatchery programs where hatchery reared fish may interbreed with naturally reared fish should consider alternative rearing environments which reduce genetic changes leading to domestication.

Workshop Discussion

Q. Was there any history of mixing with hatchery fish in the ancestry of your wild population?
A. Excellent question. There may have been some intermixing with the wild stock, but it would have been in previous generations, and was likely to be minimal.

Presentation 1.11:

Conservancy Ponds

Al Adams

Hood Canal Salmon Enhancement Group, P.O. Box 2169, Belfair, WA 98528

Synopsis

Not submitted

Workshop Discussion

No discussion was recorded

Presentation 1.12:

Comparison of Rearing Characteristics for Spring Chinook Salmon Reared In Conventional and Baffled Raceways at Willamette Hatchery, Oregon

R.D. Ewing

Biotech Research and Consulting, Inc. 2340 SE Ryan St., Corvallis, OR 97333

Synopsis

Baffled raceways have been reported to overcome two of the major difficulties with conventional raceways: water quality and the need for cleaning. These raceways are designed to be long and narrow to provide very rapid water turnover. Baffles are inserted at intervals to cause a rapid flow along the bottom of the raceway to facilitate self-cleaning. With supplemental oxygen, these raceways can support large numbers of trout with no deleterious effects.

As part of a larger study to assess the benefits of supplemental oxygen on rearing of spring chinook salmon, we examined the rearing characteristics of these fish in baffled and conventional raceways. Two sets of conventional raceways were used: group A -- those with rearing densities (kg/m3) similar to the baffled raceways, and group D -those with loads (kg/Lpm) similar to the baffled raceways.

Fall growth rates of fish in group A were significantly greater than those reared in baffled raceways in three of the four rearing years, whereas growth rates in group D were significantly greater in only one of four rearing years. When growth rates were combined for all four years, those of group A were significantly different from those of fish in baffled raceways. Average weights and lengths at release were significantly greater for fish in both groups A and D than those in baffled raceways. This was the result of significantly greater feed conversion in baffled raceways. Feed delivery time was significantly greater for baffled raceways than conventional raceways. Mortalities were significantly smaller in baffled raceways but we suspect that this was because greater bird predation occurred in the baffled raceways. When fish from all groups were taken to Marrowstone Field Station for seawater challenges, significantly more fish from baffled raceways died from bacterial kidney disease.

When metabolic activity of the fish in various raceways was examined, fish reared in baffled raceways had significantly higher oxygen consumption, higher carbon dioxide production, and higher ammonium output. No significant differences were seen in smolt indices or migration rates. However, returns from fish reared in conventional raceways were much greater than those from fish reared in baffled raceways. We conclude that spring chinook salmon are more susceptible to the influence of space limitations than the influence of water quality, unlike the rearing limitations of coho salmon and rainbow trout. Increased flow and crowded conditions of the baffled raceways reduced hatchery performance and survival of spring chinook salmon. Spring chinook salmon should therefore be reared in conventional raceways rather than baffled raceways.

Workshop Discussion
No discussion was recorded

<u>Presentation 1.13:</u>

Development of Quantitative Methods to Evaluate Behavior and Stress Susceptibility of Salmonids

Rick Barrows
US Fish and Wildlife Service, 4050 Bridges Canyon Rd., Bozeman, MT 59715

Synopsis
Not submitted

Workshop Discussion
No discussion was recorded

SESSION I: PANEL DISCUSSION

The various presenters from Session I fielded questions from the other workshop participants.

Anon. Can Steve (Schroder) or Des (Maynard) describe the methods they had used to fabricate and clean the substrate tiles used in their natural rearing studies?

Schroder. I recommend a colored concrete matrix with embedded rock, rather than the fiberglass tiles used in some early trials. Further, anyone interested in using a gravel substrate should try to obtain gravel that is as similar as possible in coloration to the gravel in the area in which the fish will be released. In that way, the fish will be color-matched to the substrate they will encounter in the stream.

Moderator. We have heard a wide variety of approaches to constructing natural rearing environments today. I am curious whether any of the panelists have heard anything today that would cause them to change their approach to someone else's. Is there any consensus about which variables may be the most important?

Adams. Personally I have learned a lot today. However, I am not sure I heard anything that, from a practical standpoint, could be incorporated into our operation in the Hood Canal area.

Anon. It was not too long ago that earthen rearing ponds were the norm in hatchery action. We then shifted away from that approach to concrete raceways and pathogen-free water for disease control. Now you're talking about shifting back in the other direction to create more natural hatchery rearing environments, and I am curious about how you would deal with the disease concerns that led us away from earthen rearing ponds in the first place?

Barrows. Speaking for our region and the Fish and Wildlife Service, the number one criteria for hatchery managers in our region has been production and cost. It has been more of an aquaculture production orientation than a stock enhancement operation. Recently, there has been more emphasis placed on fish quality, and it could be that if we move too fast, we are going to run head-on into the disease problem again.

Jateff. The same is true, historically, of hatchery production philosophy in Washington. The orientation has been toward maximum production and high densities. One of the worst things that ever happened to the hatchery program was the density study findings that said high densities produced more returning adults than low densities. That gave people an excuse to keep ponds full. If you are concerned about disease problems, one way to take care of that is to reduce densities, regardless of what type of ponds you are using.

Anon. Have any of the panelists considered using home-grown scavengers to take care of their substrate cleaning problems?

Maynard. We have considered it, but have never implemented it. I think it is an intriguing idea, but there are only so many things you can try to do in a study like this and still do them well.

SESSION II. ANTIPREDATOR CONDITIONING

Session moderator:
Steven L. Schroder
Washington Department of Fish and Wildlife, 600 Capitol Way N., Olympia, WA 98516

<u>Presentation 2.1:</u>

Predator Avoidance Training to Increase Postrelease Survival of Chinook Salmon

Desmond J. Maynard, Anita L. LaRae, Gail C. McDowell, Glen A. Snell, Thomas A. Flagg, and Conrad V.W. Mahnken
National Marine Fisheries Service, Manchester Research Station, P.O. Box 130, Manchester, WA 98353

Synopsis

Predator avoidance training may be a tool fish culturists can use to increase postrelease survival of hatchery-reared salmonids. Laboratory studies indicate salmonids observing predation on conspecifics have a higher probability of survival in subsequent predation challenges than predator-naive fish. In order to test this concept on a hatchery scale, 16,000 fall chinook salmon swim-up fry were stocked into each of six fiberglass raceways (6,000 L) equipped with predator-tight covers. Fish in three raceways were designated as controls and, prior to release, were never exposed to predacious birds or fish. Fish in three other raceways were exposed to great blue heron, hooded merganser, largemouth bass, and brown catfish predation prior to release. After exposure, tagged fish from each raceway were released into Curley Creek, a tributary stream in Puget Sound, to evaluate the effect of training on postrelease survival. Significantly ($P < 0.05$) more trained than untrained chinook salmon were recovered at a downstream weir. The higher relative recovery (26%) of trained versus untrained fish suggests enhancement and conservation hatcheries can use this approach to increase salmon postrelease survival.

Workshop Discussion

Q. Before you embarked on your predator avoidance training program, did you know there was a predator problem in the release area, and are you sure you can relate the differential survival to predation?

A. We knew we had a predation problem. That lake is loaded with largemouth bass; there are kingfishers lining both side channels, as well as great blue heron. In terms of relating the differential survival to predation, there are some assumptions in here, but there was no other variable involved that we know of.

Q. Did your control and study fish both receive the gravel treatment prior to release?

A. No. The only variable we introduced in this test was predator avoidance training. We used regular gray concrete raceways to rear the fish, although there was some algae on the walls.

Q. How much time generally elapsed between release and recapture?

A. It was generally to two to three days.

Q. At what point in the fish's life-cycle would you start expose them to predation?

A. Based on what we've seen, I would say within two weeks of when you plan to release them, and I would do it repeatedly.

Presentation 2.2:

Chemical Alarm Signals in Chinook Salmon Juveniles

Barry A. Berejikian

National Marine Fisheries Service, Manchester Research Station, P.O. Box 130, Manchester, WA 98353

Synopsis

Hatchery-reared juvenile chinook salmon were exposed to extract of conspecific tissue or to comparable stimuli from swordtails. These "injured fish" stimuli were paired with water that had contained predatory cutthroat trout. Chinook salmon receiving conspecific stimuli showed antipredator behavior (reduced food strikes, motionless behavior and deeper in water) compared to chinook salmon receiving swordtail stimuli. When the two groups of chinook salmon were tested two days later, with cutthroat trout stimulus alone, the chinook salmon that had originally received injured conspecific stimuli paired with cutthroat trout stimulus spent more time motionless than chinook salmon that had received swordtail stimuli and cutthroat trout stimulus. Ten days after the initial stimulus presentation there were no significant differences. Marked groups of chinook smolts reared in barren and complex (gravel substrate, submerged structure, overhead cover and underwater feeders) tanks were treated with control (distilled water) stimuli or with injured conspecific stimuli paired with cutthroat trout stimuli and released into a natural stream. The results suggest that the complex rearing treatment had a negative effect on survival that was compensated for by the application of the paired stimuli. Chinook salmon, like rainbow trout, show antipredator behavior in response to chemical stimuli from injured conspecifics and learn predator recognition when such stimuli are paired with predator odor, improving survival in the wild.

Workshop Discussion

Q. For smolts migrating through high flows and turbid water, do you think chemical alarm signals for predator avoidance play much of a role?

A. For the particular circumstance, who knows? It appears that these fish have an innate ability to recognize predators, but that innate behavioral response needs to be triggered through some predation event. All I am really saying hear is that this may be a way to trigger that innate behavior response -- under various conditions, it may or may not have a benefit.

Q. You are calling this communication, yet you are taking the fish and grinding them up. Is it not possible that what you are seeing is simply a response to an odor profile, rather than to a pheromone that is being used to communicate?

A. That is a good point. As a population the fish are communicating, but they may not actually be generating a pheromone. In other fish species, a pheromone exists in the epidermal club cells during certain life history stages; if those cells are mechanically ruptured, that pheromone is released.

Presentation 2.3:

The Effects of Hatchery and Wild Ancestry on the Relative Ability of Chickamin River Chinook Salmon to Avoid a Natural Predator

John Joyce, Alex Wertheimer, and John Thedinga
National Marine Fisheries Service, Alaska Fisheries Science Center, Auke Bay Laboratory, 11305 Glacier Highway Juneau, AK 99801

Synopsis

Increasing concern has been expressed about the genetic effects of cultured salmonid fishes on natural populations. The displacement of native stocks from massive introduction of exotic stocks is an extreme example of such effects. Avoidance of such extreme outcomes was one of the reasons for the establishment of a genetic management policy for the State of Alaska. However, domestication within the hatchery may still cause divergence from the wild population. The degree to which domestication causes changes and fitness is controversial; the evidence is often circumstantial, and based on studies that were not designed to examine divergence from a common donor stock, or that separate genetic effects from the effects of environmental rearing conditions. A few studies have shown that domestication can cause genetic differences relative to the donor natural stock in behavioral characteristics, and in growth and survival of juveniles. Because of the great interest in minimizing genetic impacts of enhancement programs on natural populations, and of using enhancement technology to restore depressed or endangered natural populations, more information is needed on the degree to which domestication occurs in different species of Pacific salmon; the generation time for effects to be observed; and the generation time for effects to be removed by natural selection.

This study is one of a suite of research projects examining domestication in a stock of Alaska spring chinook. These fish commonly rear in mainland rivers and migrate as age- 1 smolts at 66 g in size. They are exposed to predation during rearing by other fishes, especially Dolly Varden charr. When cultured to age-1 smolts in hatcheries, the fish are typically released at sizes of 15-40 g. These large smolts have been protected from predation during their freshwater rearing stage, and also avoid many of the natural predators their wild counterparts are exposed to in the marine environment because of their much larger size at release. Thus the hatchery environment provides the opportunity for domestication effects on predator avoidance behavior.

Objectives

(i) Assess relative predator avoidance of F1 chinook salmon fry from wild and hatchery populations of common ancestry.
(ii) Assess the effect of hybridization of hatchery and wild populations of common ancestry on relative predator avoidance.

Methods

Study populations Chinook salmon fry for this experiment originated from the Chickamin River on the mainland coast of southern Southeast Alaska. Returning spawners were taken from the Barrier Creek tributary of the Chickamin, or from the south fork of the Chickamin River itself within 2 km of Barrier Creek. In 1976, a hatchery population of these fish was established at Little Port Walter on Baranof Island using gametes from 8 females and 9 males. Smolts from this transplant were tagged with stock-specific coded-wire tags; only spawners identified by tags as Chickamin origin were used to maintain the brood line. In 1996, fourth-generation progeny returned to LPW from the original transplant. Wild gametes were again obtained in 1996 from the same spawning area on the Chickamin River. Unfertilized gametes from five females and five males were transported by helicopter to LPW on August 12. Reciprocal crosses were made with five female and five male hatchery fish (five 2 × 2 reciprocal crosses), resulting in four categories of progeny: 1) wild male × wild female (WW); 2) wild male × hatchery female (WH); 3) hatchery male × wild female (HW); and 4) hatchery male × hatchery female (HH).

Fertilized eggs were incubated in Heath trays at the LPW facility. Fry will be ponded from the incubators in April 1997. Fry from each cross will be pooled, and a subsample of fry from each cross will be taken for use in this experiment.

The predator population for the experiment will be Dolly Varden char collected as they emigrate from Auke Lake. Salmonid outmigrants from the Auke Lake watershed are routinely enumerated as they pass through a sampling weir on Auke Creek. In 1996, 11,323 Dolly Varden emigrated through Auke Creek weir, primarily in April and May. Approximately 300 will be retained for this experiment in 1997. All Dolly Varden will be returned to Auke Creek following the experiment.

The experiments will be carried out at the Auke Creek Hatchery. Chinook fry from the four crosses of wild and hatchery gametes will be transported from LPW to Auke Creek, held in separate tanks, and fed until tested. None of these fry will be released at Auke Creek; all fry surviving the experimental trials will be destroyed.

Dolly Varden collected at the Auke Creek weir will be held in a 3000 l fiberglass tank receiving 50 l/m single-pass freshwater. These fish will be conditioned to eat fry by feeding them newly emerged chinook fry (Unuk stock) collected at Little Port Walter in April and stored frozen. Pre-experiment trials will be used to determine the size of Dolly Varden appropriate to use in the predation tests. The desired predator is one that can eat 2-4 chinook fry in 4-8 hrs.

Predator avoidance trials will be held in 85 1 fiberglass tanks. A total of 24 tanks will be used. Each tank will have approximately 8 L/min single -pass water flow. The tank array will be surrounded on all sides by black visqueen sheeting with viewing slots to allow observation of the trials without disturbance. Three series of trials will be conducted: 1) naive hatchery and naive wild fry; 2) experienced hatchery and experienced wild fry; and 3) experienced hatchery and naive wild fry.

Testing of naive fry will include comparisons of WW fry with the other three crosses. Fry will be removed from each population, anaesthetized with MS222, photographed for later morphometric and color analysis, and measured. For each trial, three fry will be specifically selected from the WW and the other cross tested such that the range in fork length within a test chamber is not more than 1.5 mm, and each individual from one cross had a counterpart from the other cross that did not differ by more than 0.5 mm.

Each group of six fry to be tested will be put into an individual tank, and allowed to acclimate at least 16 hr before a Dolly Varden is introduced into the tank at approximately 08:00. The tanks will be kept under constant surveillance, and the predator removed after 2-3 fry were eaten. The surviving fry will then be removed and examined under ultraviolet light to identify the cross population. A minimum of 50 trials will be conducted for each cross comparison. Because only 24 tanks are available, 8 series of tests will be needed to complete the trials.

The Wilcoxin signed-rank test for paired differences will be used to compare the number of fry eaten from each cross, with the Z statistic corrected for ties. For the naive/naive experiment, the test value for a Type 1 error of P = 0.05 will also be corrected for multiple comparisons with a control (WW) population.

Workshop Discussion

Q. Early in your presentation, you said the size distribution among the groups was quite different. Any idea why?

A. I think it mainly had to do with the variability in egg size among the wild females. One of the concerns we had in trying to do this type of study was the amount of variability we would see at a family or an individual level, and how that might contribute to apparent differences we might

Q. Comment. I have looked at size variability in Yakima spring chinook, and found a 100-fold difference in egg size among females within that population. Within a female, however, the coefficient of variation averages only 1.5%-2%. Age at maturation can also be a factor in egg size variability.

SESSION II: PANEL DISCUSSION

The various presenters from Session II fielded questions from the other workshop participants.

Berejikian. I have a question for John (Joyce). We did some of work looking at the differences between wild and hatchery steelhead stocks, and hypothesized a mechanism to explain the differences in vulnerability to predation. Essentially, hatchery steelhead never encounter freshwater benthic predators, so there is a potential for relaxed selection. Do you think a similar mechanism may be at work in the chinook stocks you've worked with?

Joyce. I really cannot speculate about why there might be a difference in predation vulnerability between the wild and hatchery chinook.

Brannon. Des (Maynard) and Steve (Schroder) talked about the duration of conditioning, but in a natural setting, you would think that the imprinting vulnerability of fry would be pretty high. In terms of long-lasting behavior patterns, you would think that the fry stage would be the optimum one for predator avoidance training. What are your thoughts on that?

Maynard. Intuitively, I would say that if they do not experience predator conditioning frequently, and near the time of release, they will probably be vulnerable. There are so many other stimuli these fish are experiencing - including the way they are fed - that could potentially be deconditioning that response. I am just not sure how long the predator avoidance response will stay with these fish. It could be that if these fish are conditioned as fry, you will see a really good response. At this point, however, we just don't know the answer to that question.

Berejikian. I think I have to respectfully disagree with Des (Maynard) on this one. From the literature I have read these predator avoidance mechanisms are innate. They simply need to be triggered in order to become a fixed action pattern. The fish are predator-wise after that point. My thinking it is that it does not take long to teach if these fish to avoid predators, and once they have learned, they have learned.

Brannon. I think there has always been a tendency to underestimate the profound impact of predation. But when you look at night-time emergence and night-time migration, that tells me there has been a very strong selective force at work among these fish. It also argues that fry are very receptive to this stimulus, and to me, that suggest that the fry stage is the best one to perform this type of conditioning. Perhaps what we need to try is periodic conditioning and exposure to predators at various life stages, as Steve (Schroder) suggested.

SESSION III. FEEDING, GROWH AND RELEASE ALTERNATIVES

Session moderator:
Ernest L. Brannon
University of Idaho, Moscow, ID 83843

Presentation 3.1:

The Effects of Live Food Supplementation on Chinook Salmon Foraging

Desmond J. Maynard, Gail C. McDowell, Glen A. Snell, Eugene P. Tezak, Thomas A. Flagg, and Conrad V.W. Mahnken
National Marine Fisheries Service, Manchester Research Station, P.O. Box 130, Manchester, WA 98353

Synopsis

Theoretically, the postrelease foraging ability of hatchery-reared salmon may be improved by adding live foods to their diets. It is known that during their first few weeks after release, hatchery-reared salmonids appear to be starving and have much less digestible matter in their stomachs than wild-reared fish. This may result from pellet reared salmonids having no meaningful opportunity to develop their natural hunting skills. The addition of live food to the diet may provide salmon the opportunity to develop these critical predatory skills prior to release. The following studies investigate if the addition of live food in the diet improves the foraging behavior and success of hatchery-reared chinook salmon.

The first study investigated how supplementing the diet with live-food alters fall chinook salmon foraging behavior. The study was initiated by establishing six replicate groups of fry reared in circular tanks (2.4 m diameter). In three tanks fry received a standard pellet diet, while those in three other tanks were given the opportunity to forage on live prey (mysids, mosquito larvae, chironomid larvae, and daphnia) prior to a daily ration of pellets. When the foraging ability of individual fish was examined in laboratory test arenas (200 L), the trained fish fed on twice the number of familiar (chironomids) and novel prey (mayfly larvae) as untrained fish. This suggests that supplementing the diet with live-food increases foraging ability.

In another study, 24 replicate groups of yearling spring chinook salmon were held in tanks (400 L) for the final months of freshwater rearing. The study utilized a two way experimental design, with the presence and absence of gravel substrate as one factor and live food supplemented and non-supplemented diets as the other. On any given day, fish in all 24 tanks received an equal volume of feed pellets. In addition to pellets, fish in the 12 live food supplemented tanks (6-bare bottom and 6-gravel bottom tanks) were given a ration of brine shrimp or tubifex worms. At the end of the experimental rearing period a sub-sample of fish from all 24 tanks were challenged to forage for a week on naturally available prey in both freshwater (cages in Union River) and marine (artificial marine pond) enclosures. The fish were later sacrificed and stomach contents sampled. Many of the fish had empty or near empty stomachs, indicating they were not feeding well. In the marine enclosure, pellet-reared fish had significantly more material in their stomachs than live-food reared salmon. In the freshwater enclosure there was no significant difference detected, but pellet-reared fish had the greater weight of material in their stomachs. The presence or absence of gravel did not significantly effect the weight of material in salmon stomachs. The data suggests that supplementing the diet with live feed will not improve salmon foraging success.

The third study investigated how a total live food diet effects salmon foraging behavior and success. Swim-up fall chinook fry from Bingham Creek Hatchery (WDFW) were systematically divided up into six equal lots. Fish in three lots were reared exclusively on a standard prepared semi-moist diet, and fish in three other groups were fed only a live food diet, primarily consisting of brine shrimp, blackworms, and glassworms. The foraging success of the fish was evaluated by stocking them in cages submersed in Bingham Creek, and allowing them to forage for one week. The preliminary data indicated that live-food-reared fish had more material in their guts than pellet-reared fish. As in the second experiment (above) many fish had little material in their gut.

In conclusion live food supplemented diets can sometimes improve chinook salmon foraging ability. However, the magnitude of this improvement is presently insufficient to justify the added investment required to rear production fish on live food diets.

Workshop Discussion
No discussion was recorded

<u>Presentation 3.2:</u>

Redfish Lake Sockeye Live Food Training Study

J. Pravecek
Idaho Department of Fish and Game, 1414 E. Locust, Nampa, ID 83669

Synopsis
Not Submitted

Workshop Discussion
No discussion was recorded

<u>Presentation 3.3:</u>

Effect of Diet Formulation on Dorsal Fin Erosion in Rainbow Trout

Richard Barrows
U.S. Fish and Wildlife Service, 4050 Bridges Canyon Rd. Bozeman, MT 59715

Synopsis
Not Submitted

Workshop Discussion

Q. Your work implies that early feeding of this alternative diet is important. Have you given any thought to the possibility of starting them on the enhanced diet early on, then stopping it at some point?

A. I think that as far as fin development that would probably work. I know of one commercial trout operation where the fish were started on the enhanced diet, then switched over to a commercial diet and raised until they were 12 inches long. The fish looked great, and fin quality was excellent. However, from an immune system standpoint, I am not sure how good an idea that would be.

Q. Do you think fin nipping may be a factor in fin erosion?

A. Yes. We have just finished another study, in which fish fed a regulation fish meal-based diet were raised individually, and showed perfect fin development. That tells me that fin erosion is an effect of fish interaction. However, even in crowded tanks, when the fish are fed the enhanced diet, the fins stay in good shape.

<u>Presentation 3.4:</u>

The Effect of Automated Subsurface Feeders on the Behavior and Predator Vulnerability of Fall Chinook Salmon

Desmond J. Maynard, James L. Hackett, Michael Wastel, Anita L. LaRae,
Gail C. McDowell, Thomas A. Flagg, and Conrad V.W. Mahnken

National Marine Fisheries Service, Manchester Research Station, P.O. Box 130, Manchester, WA 98353

Synopsis

The study compared in-culture survival, behavior, and predator vulnerability of fall chinook salmon fed by an automated subsurface feed delivery system to those fed by hand. The experimental rearing was conducted with six fiberglass raceways (6,000 L) each stocked with 4,800 fall chinook salmon fry. An automated underwater feed delivery system was used to feed fish in three of the raceways, while control fish in three other raceways were fed by hand. Except for feeding method fish in both treatments were reared identically, following standard salmon culture protocols.

The fish in the hand fed raceways rapidly became conditioned to swim to people and swarm at the surface when humans approached the raceway. In contrast, fish fed by automated feed delivery system held position and remained near the raceway bottom when people approached the tank. Underwater video observations made during non-feeding periods demonstrated that the depth preference of fish in both treatments was similar and that fall chinook salmon have an innate benthic orientation. Observations on the depth preference of fish removed from their rearing environment and tested in laboratory arenas confirmed this innate depth preference with fish from both treatments generally residing within 4-cm of the bottom.

The fish were challenged to avoid merganser predation in test arenas that simulated a natural stream environment. In one series of tests the fish were placed in adjoining test arenas

(one treatment/arena) and the birds given the opportunity to demonstrate which arena contained fish that attracted their attention most often. In a second series, the fish were tagged and both treatments placed in the same predation bioassay arena. In both series the fish from the two treatments exhibited nearly identical survival, indicating that the way they were fed did not effect their vulnerability to predators.

The fright response of the fish to visual stimuli at the surface was investigated by observing their response to a model of a great blue heron, a shovel, and a human standing alongside each raceway. The response of the fish in both treatments to the two novel stimuli (a great blue heron model and a shovel) was identical. However, only fish fed by the automatic subsurface feed delivery system exhibited a strong fright response to the image of a human standing beside the raceway. Hand-fed fish readily swam over to the image looking for food. This suggests that hand feeding only conditions fall chinook salmon to approach the image of humans, and does not put fish at a greater postrelease predation risk.

Workshop Discussion

Q. Have you seen any difference in predator avoidance behavior between groups of chinook fed using surface automatic feeders and subsurface automatic feeders?

A. I think if you use an automatic surface feeder, as opposed to hand-feeding these fish, the change in avoidance would be about the same as you would see if you used a subsurface feeder. The change from hand-feeding to automatic feeding is the main factor in preserving the predator avoidance behavior.

Presentation 3.5:

Juvenile Development and Smoltification of Spring Chinook Salmon at Pelton Ladder, Oregon: An Empirically-Derived Natural Rearing

Walton W. Dickhoff[1], Brian R. Beckman[1], Donald A. Larsen[1], and Cameron Sharpe[2]

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[2]*Washington Department of Fish and Wildlife, Kelso, WA*

Synopsis

Rearing conditions vary for salmonids in public salmon enhancement hatcheries due to differences in water source (ground versus surface), water temperature, raceway design, rearing densities, and feeding rates, among other environmental factors and husbandry techniques. Although general guidelines for rearing salmonids are available, sometimes the specific rearing protocols have been developed empirically over decades of experience by hatchery staff based on optimal fish health and smolt-to-adult survival. In our studies of hatchery spring chinook salmon smolt quality in the Columbia River Basin, an interesting case study is presented by the Round Butte Hatchery/Pelton Ladder rearing facilities (Oregon Department of Fish and Wildlife). Generally higher smolt-to-adult survival is observed from fish reared in Pelton Ladder compared to Round Butte Hatchery despite the fact that smolts from Round Butte hatchery are generally larger than Pelton Ladder smolts. Smolt-to-adult survival for brood years 1979-83 averaged

0.29% for Round Butte and 1.26% for Pelton Ladder; smolt-to-adult survival in our study for brood years 1988-90 averaged 0.69% for Round Butte and 1.15% for Pelton Ladder. The rearing environment in Pelton Ladder may contain more natural elements and may serve as a heuristic model for developing natural-rearing technology. We present data on the physiology and morphology of juvenile salmon reared in the Round Butte Hatchery, Pelton Ladder, and Warm Spring National Fish Hatchery (also on the Deschutes River), examine smolt-to-adult survival of released fish, and speculate on the significance of differences in these facilities.

Round Butte hatchery is located at the base of Round Butte Dam. Water temperature is a relatively constant 10° C (50° F) and fish are reared in Burrows-type concrete raceways (4.9 m × 23.1 m; 16 × 75 ft) with a water depth of 1.0 to 1.3 m. Rearing density in the Burrows ponds ranged from 0.39 to 1.1 lb/cu ft. Water flow rate was approximately 500 gpm until the last two months of rearing when it was increased to 900 gpm. Fish are transported by truck to Pelton Reregulating Dam for release.

Pelton Ladder was built as an adult fish passage facility, but is not used for adult passage. The ladder runs downstream 4.5 km from Pelton Dam to Pelton Reregulating Dam; the lower 0.5 km has been used as a satellite juvenile rearing facility for Round Butte Hatchery since 1979. Underyearling fish are transferred from the hatchery to the ladder in late October or November. The concrete ladder is 3.1 m (10 ft) wide with water depth of 2.5 m (8 ft) and is divided into approximately 98.5 m (320 ft) sections. Water flow is approximately 480 gpm. Water temperature ranges seasonally from 4 to 13° C. Maximal rearing density was approximately 0.3 lb./cu ft. Fish in Pelton Ladder have access to natural foods in the water. Furthermore, the rearing container is darker and larger than the Burrows ponds at the hatchery. Fish are allowed to leave the ladder volitionally beginning on the release date.

Fish were sampled bi-monthly beginning as underyearlings in September and continuing until release in April. Analysis included length, weight, condition factor, plasma hormone concentration thyroxine, cortisol and insulin-like growth factor-I (IGFI), stress challenge, gill Na+K+ ATPase activity and liver glycogen concentration. Fish from Round Butte hatchery were larger than fish from Pelton Ladder due to relatively greater growth of Round Butte fish during the winter. Pelton ladder fish showed the greatest growth in spring, a pattern that matches that of naturally reared fish. Gill ATPase activities and plasma IGF-I showed earlier elevations in round Butte fish compared to Pelton fish. However, despite the smaller size of Pelton fish, most of the physiological measures of smolt quality were similar to Round Butte fish at the time of release. A comparison of Round Butte, Pelton and Warm Springs Hatchery fish showed significant correlations between smolt-to-adult survival and February to April growth rate, gill ATPase activity and plasma IGF-I concentration. These results suggest that spring growth rates may be important for enhancing postrelease survival. Furthermore, other differences in the rearing environment between Round Butte Hatchery and Pelton Ladder may enhance postrelease survival. These differences include seasonally and daily fluctuating water temperature, lower density, access to natural food, reduced incident light, volitional release, and a larger rearing container, among other factors.

Workshop Discussion

Q. My understanding is that some of the survival benefits you describe may have dissipated as rearing densities have increased at Pelton.

A. That is possible, although I don't know whether it is in fact true. But it is a huge facility, and there is some additional capacity there.

Q. You started off your presentation showing a high correlation between growth rate and survival, but also highlighted a lot of other variables - size, stock, feed, rearing container type, density etc. I am wondering whether a number of these factors are not interacting to produce the kinds of smolt-to-adult survival results you have described, and whether it is appropriate to conclude that growth rate is the key.

A. The growth rate correlations are very strong. It is not really a conclusion, it is a result. I agree that all of the other factors you mentioned contribute to growth rate, and that growth rate is linked, mechanistically, to the parr-smolt transformation.

Q. Do you think an increased feeding rate might increase postrelease survival?

A. When we have increased spring chinook rations during the spring period, we have found increased in-stream survival and rate of migration. We are currently testing this concept on a production scale at Young's Bay.

Q. In your research, can you separate growth and length from growth and weight?

A. We have found correlations with both. Growth and length is linked to protein anabolics, while growth and weight is influenced by body lipid level; the bottom line is that both of them show correlations.

Q. And did you see a decline in body lipid levels even while you were force-feeding your fish?

A. Yes.

<u>Presentation 3.6:</u>

Growth, Metabolism and Smoltification of Naturally-Rearing Yakima River Spring Chinook Salmon Juveniles

Brian R. Beckman[1], Donald A. Larsen[1], Walton W. Dickhoff[1], and Cameron Sharpe[2]

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Synopsis

The goal of natural rearing systems is to improve smolt to adult survival of hatchery salmon by rearing them so that they are phenotypically similar to wild fish. However, many aspects of wild parr and smolt biology remain undescribed. In this work we characterize the physiological and endocrine patterns associated with growth and smoltification in wild spring chinook salmon juveniles from the Yakima River, a tributary of the Columbia River located in central Washington. We thus provide a template for growth and smoltification of juvenile chinook salmon in natural rearing systems.

Fish were sampled from June, approximately three months post-emergence, through May of the following year when fish initiated smolt out-migration as yearlings. Sampling was

conducted with electro-shock gear and seines over a 200-km stretch of river. Fish were sampled in both up-river reaches, where fish resided for over a year, and lower river reaches, to which fish migrated in the fall and over-wintered, before smolt out-migration the following spring. Migrating smolts were also collected at a lower river fish bypass facility at Prosser Dam during the out-migration period.

Distinct, dynamic changes were found in each of the parameters examined, which included, fish size, condition factor, body silvering, stomach fullness, liver glycogen, body lipid, plasma thyroxine (T4), plasma insulin-like growth factor I (IGF-I), and gill Na+K+ -ATPase. Elevated growth rates were evident from June through October and from February through April, little or no growth was observed from late fall through the winter months. Liver glycogen and body lipid levels were elevated through the early fall, declined through November-December to low levels by February. Significant increases in both glycogen and lipid were found in late February and March, however, these stores were significantly depleted in out-migrating smolts. Plasma T4 and IGF-I values increased in the spring coincident with smoltification. Very high values for IGF-I were found in out-migrating smolts. Gill Na+K+-ATPase activities showed a distinct increase in the spring, coincident with smoltification. Highest values were found in migrating smolts.

Dramatic changes were observed in metabolic energy storage (anabolism) and energy mobilization (catabolism). A distinct anabolic to catabolic shift was found in November and a catabolic to anabolic shift in February. Low feeding rates (inferred from stomach fullness data), growth rates, and reduced metabolic reserves were observed in winter months. Increased feeding rates, condition factor, liver glycogen, body lipid, and plasma IGF-I levels were all found in February-March, when water temperatures were still relatively cold (5° C). Studies of fish reared in a hatchery or laboratory have not shown as dramatic metabolic changes as seen in wild fish. We hypothesize that these physiological changes are due to a combination of low temperature and food supply in the winter, coupled with increasing temperatures and food in the spring, conditions seldom found in hatchery environments. We suggest that the dynamic anabolic rebound found in February in wild fish may have synergistic effects with the endocrine process of smoltification, resulting in more synchronized physiological changes and a superior smolt.

Workshop Discussion

Q. Comment. A number of years ago we had two big surface-fed coho ponds at the Humptulips hatchery. The fish were fed by demand feeders, and we allowed the fish in one pond to eat whenever they wanted to. During the winter, they only fed themselves at about 0.7% body weight, which is probably less than our hatchery staff would have fed them if it had been up to them. I think that correlates up pretty well with what you've been talking about in your presentation.

Q. What were your temperatures?

A. It is a coastal stream, so temperatures probably varied between 41 - 47 degrees during the winter period.

Q. Comment. Another interesting thing is the fact that hatchery personnel observed that the coho would begin eating at certain times of the day. That was the cue for them to grab their

steelhead rods and go out fishing, and they always caught fish that time of day.
A. It is definitely true that wild fish have natural feeding patterns, but that really is not something we have tried to mimic in the hatchery.
Q. There was an apparent decline in the upper size of your wild fish from the fall to the spring. There have been some observations of larger fish moving out as smolts during the fall, and I was curious whether you have seen any evidence of that tendency in your fish?
A. The Yakima fish certainly move around a lot in the fall. There is a big migration from the upper reaches down to the area below Yakima.

Presentation 3.7:

Do We Need to Feed Hatchery Fish in the Winter? The effect of Low Temperature and Starvation on Growth, Metabolism and Smoltification of Coho Salmon

Donald A. Larsen, Brian R. Beckman, and Walton W. Dickhoff
Integrative Fish Biology Program, Northwest Fisheries Science Center
National Marine Fisheries Service, F/NWC1, 2725 Montlake Blvd. E, Seattle WA 98112

Synopsis

Recent research in our laboratory has provided evidence that both successful hatchery smolts and wild smolts display a more dramatic seasonal dynamic in growth, metabolism and endocrine physiology prior to smolting, compared with less successful hatchery fish. This dynamic is characterized by low growth in winter and high growth in the spring, prior to outmigration, and dramatic changes in various physiological parameters including condition factor, liver glycogen, body lipid, plasma thyroxine (T4), plasma insulin-like growth factor I (IGF-I) and gill Na+K+ATPase. In the transition from autumn to winter, wild fish shift from an anabolic (energy storage) to a catabolic (energy mobilization) state characterized by decreased feeding, a reduction in metabolic reserves and reduced growth rate. In the early spring the pattern is reversed by a switch from catabolism to anabolism with feeding, energy stores, and growth rate all increasing prior to outmigration. We have hypothesized that this physiological dynamic is an essential process seldom experienced by hatchery fish which are commonly fed throughout the winter months.

The objective of this study was to examine the effect of winter feeding and starvation, under both high (10° C) and low (3° C) temperature, on growth and physiology of coho salmon prior to and during smoltification (Jan-May). The temperatures were intended to approximate typical winter hatchery ground water (1O° C) and surface water (3° C) conditions. The treatments consisted of the following groups: Warm-Fed (WF), Warm-Not Fed (WNF) Cold-Fed (CF) or Cold-Not Fed (CNF). During the five months (Oct-Feb) prior to smoltification fish were either fed (at the manufacturers specified rate at each temperature 1.5% BW-10° C, 0.6% BW-3° C) or starved during a two month period (Jan-Feb). During March through May all groups were reared at 10° C and fed at 1.5% BW. Throughout the investigation the following parameters were measured: length, weight, instantaneous growth, hepatosomatic index, body lipid, liver glycogen, gill Na+K+ATPase and plasma levels of IGF-I, T4 and insulin.

The results show that WF fish grew continuously throughout the winter and were larger than the other treatments. All other groups were smaller and showed depressed growth during Jan and Feb, including the CF group, despite the fact that it was fed. Among the physiological parameters which have been measured to date; condition factor, hepatosomatic index, plasma IGF-1, and liver glycogen were all highest in the WF fish and depressed in the WNF fish during the winter. The CF and CNF groups were intermediate. However, during the spring, when all groups were fed and returned to the warm temperature, the previously starved groups (WNF and CNF) showed very similar, dynamic changes in most physiological parameters while the continuously fed groups (WF and CF) displayed less change.

The data are not yet complete, and we do not know whether winter-starved fish show smolt development equivalent to the winter-fed fish. The fish were not tested for migratory performance. However, the growth and physiological profiles of the winter-starved animals, at both high and low temperature, more closely resembled that of wild salmonids. Future efforts to rear more "wild-like" salmonids under hatchery conditions should recognize the importance of the interaction between season, temperature and feeding on the physiology of the fish being released.

Workshop Discussion

Q. Have you noticed any differences in mortality or fish health between groups?

A. None at all.

Q. And of what type of food did you feed them before and during the study?

A. Biodiet.

Q. Are you planning to repeat this test with spring chinook? Because historically we have seen a lot of problems with gray tail and fin erosion in spring chinook.

A. Yes.

Q. How did you go about deciding what your dormancy period would be?

A. To be honest it was a shot in the dark. If you look at the data from wild fish, you would say that the dormancy period is about the four months long, starting in late October and going through March. We decided to try a couple of months, and we think the spring chinook experiment will tell us more.

<u>Presentation 3.8:</u>

Evaluation of Chinook Salmon Hatchery Release Strategies: Matching Seasonal Migration Patterns of Natural Fish in Lookingglass Creek

Richard Carmichael

Oregon Department of Fish and Wildlife, 211 Inlow Hall EOU 1410, Lagrande, OR 97850

Synopsis

Not Submitted

Workshop Discussion

Q. What were the differences in release strategy between the acclimated and non-acclimated groups?

A. There was no difference.

Q. What about migration timing?

A. For the Catherine Creek spring chinook stock in the upper Grande Ronde basin, many of the larger fish (about 50% of the population) tend to move down out of the upper basin in the fall. Yet in the Upper Grande Ronde basin as a whole, only about 10% of the juveniles move down into the lower valley to over-winter, which I find very interesting. In other words, we are seeing some very different migration patterns even in populations with similar juvenile life-histories.

Presentation 3.9:

Performance of Spring Chinook Salmon Juveniles Reared in Elevated Flow Conditions – Do Active Fish Make Better Smolts?

Steven Parker

Oregon Department of Fish and Wildlife, 211 Inlow Hall 1410 "L", Lagrande, OR 97850

Synopsis

Not Submitted

Workshop Discussion

Q. You talked about possible behavioral modifications in response to changes in current velocity. Do you have any plans to study that at the hatchery, or would it be possible for you to provide fish to researchers who were interested in taking a look at things like habitat selection and social behavior under elevated flow conditions?

A. I would like to see some of that work get done. Exercise has been shown to have some pretty dramatic effects on the physiology and behavior of fish, but it would be useful to know more about exactly where those effects are coming from, and what physical parameters are needed to create them.

Q. In the wild it is pretty unusual for fish to be exposed to uniform currents. Have you considered the possibility of modifying raceways so that fish can spend some time in faster currents, then seek slower-current refugia, rather than simply swimming their fins off all the time?

A. Actually we are seeing some of that already. The water really shoots out at the upper end of the raceway, then starts to slow down as it runs into the main body of water, and you get a big sinusoidal wave going down the raceways. There are backspins, eddies and algae growth that allow fish to get out of the current if they want to.

Moderator. I think Barry (Berejikian) made a good point about collaborative work between some of the researchers present here today. The workshop organizers were really hoping to encourage those kinds of connections and interactions, because if we do not do more of that kind of collaborative work, we are probably going to fall behind on the funding curve. Also a word of caution related to this particular research area. A number of years ago, some experiments were done to look at fish stamina as a predictor of future performance and survival, in which fish were transferred from various parts of the basin to Cultus Lake Hatchery for testing. Those that did not do as well on the stamina test were considered to be inferior fish, but the researchers were surprised when, a few years later, the “inferior” fish from one particular hatchery turned out to be superior in performance to all of the other groups. The problem was that, when these fish were introduced to a strange water source, they rejected it, and that had an influence on their behavior. My point is simply that we have to be careful how we interpret our results when fish are transferred from one water source to another.

SESSION III: PANEL DISCUSSION

The Session III presenters spent a few minutes fielding questions from the workshop audience.

Anon. With respect to the work that is being done on reducing feed rations during the winter, I was curious how much work has been done to look at the effects of reduced photoperiod, as well as reduced temperature, on feeding behavior?

Larson. The data so far does suggest that photoperiod is also having an effect. Just following the feed charts with respect to temperature may not be the whole story, and I think it is fair to say that photoperiod may not have been given the consideration it deserves during the research to date.

Carmichael. A couple of interesting observations from our life history studies. First, that we see wild spring chinook in the Grande Ronde that are actively feeding at temperatures down to 2° C. We are also seeing migration continue through the winter at that temperature. In other words, those wild chinook aren't just sitting on the gravel through the winter. I would add that, in our wild fish, we have found food in their stomachs at all times of the year. They were not as full during the dead of winter, but they did have food in their bellies.

Anon. I had a question for Des (Maynard) about underwater feeding. You have been doing this research for a number of years now and, based on what I have heard over the last two days, it does not sound like you can demonstrate a clear-cut advantage to underwater feeding. At what point do you put this line of research to rest?

Maynard. When I run out of ideas and ways to refine a study, that is when I put it to rest. For at least some of the stuff we are doing with automatic feeders, that is something I have put to rest, in my mind at least. When we see something that works over and over again, such as the semi-natural habitat and predator training work we have been doing, I say, let us start using those techniques in a hatchery setting. How long it will take for these techniques to be widely implemented, however, is up to the managers. But if you've got a technique that has worked consistently well over several trials, that shows only positive effects and no negative effects, that is something you may want to consider implementing.

Carmichael. It is pretty clear from the data that there is a survival advantage for natural fish over hatchery fish. I think the secret, for us, is to take a look at the systems we are working in, and determine what factors are causing hatchery fish to die at a higher rate than natural fish. Every hatchery situation is different, and not all of the research we have heard about over the last two days is going to be useful to every hatchery's situation.

Parker. Aside from looking for variables in nature that we want to try to mimic, there are a number of variables that have been shown to work in certain situations. The question becomes, how do you modify your hatchery situation to utilize that information? The problem is, you run into a lot of walls very quickly when you start suggesting major modifications to a long-term hatchery operation.

Fuss. I am curious whether anyone has an opinion about whether all of the work we are doing with seminatural rearing systems, predator training and other naturally-based rearing techniques is putting more selection, or less selection, on the fry-to-smolt stage, in comparison to what goes on in a natural system? The reason I ask is that one of the biggest criticisms of hatchery programs in general is that we are removing the selective pressures from the early stages of rearing. My question is, are we removing even more selective pressure by developing these

natural rearing systems, or are we increasing selective pressure? Because in natural systems, at least for anadromous fish, most of the selection occurs during the emergence-to-smolt stage.
Berejikian. I guess that is the next big study. We have discussed the theoretical possibility that a seminatural environment might reduce natural selection in the hatchery. The only way to discover the answer would be to do that experiment, and it will not be an easy one. Until that study is done, all we can really do is theorize about what a NATURES-type environment is doing to the fish.
Berejikian. My question for Rich (Carmichael) relates to in-stream survival. If the mortality that is occurring between the time your chinook are released and the time they arrive at the dams may not be due to predation, but due to some other factor, what other mortality mechanism could explain the fact that these fish disappear within a few days or weeks?
Carmichael. I really do not know, and I would not rule out predation as one of the major factors. But when you look at the migration conditions these fish encounter in the Grande Ronde, such as the high flow, turbidity and low temperature, then I just do not think predators would be very effective under those conditions. A lot of mortality is also occurring at the head of the reservoir, and we know there is predation at work there. In other words, it is not simply a stream effect, there are reservoir effects at work as well.

SESSION IV. EXPERIMENTAL DESIGN

Session moderator:

Robert N. Iwamoto
REUT Division, Northwest Fisheries Science Center,
National Marine Fisheries Service, F/NWC1, 2725 Montlake Blvd., Seattle, WA 98112

<u>Presentation 4.1:</u>

Monte Carlo Simulations to Determine the Power of Experiments Comparing Rearing Treatments

Curtis M. Knudsen, Craig A. Busack, and Annette Hoffmann
Washington Department of Fish and Wildlife, 600 Capitol Way N., Olympia, WA 98501

Synopsis

Choosing an experimental design with sufficient statistical power to detect differences in survival among experimental populations should be one of the first steps in study development. By determining that an experimental design has sufficient statistical power one can be certain *a priori* that the design will allow detection of a predetermined difference in survival given set values for α (Type I error) and β (Type II error) with the caveat that the assumptions made are not grossly violated. If a design is statistically under powered then after the study is completed it is not possible to interpret results that do not reject the null hypothesis. Was the failure to reject due to no difference among populations or due to a Type II error where by chance we fail to reject when in fact there is a true difference?

In developing the experimental design of the Yakima Fishery Project (YFP) we wrote a number of Monte Carlo computer models that allowed the user to specify physical and biological parameters in a postrelease survival experiment and then estimated the resulting statistical power of a randomized-block ANOVA. The user specified parameters include the basic physical layout during rearing (number of release sites, number of replicate ponds per release site), the population size per pond at release, expected postrelease survivals, expected survival difference between treatment and control populations, the magnitude of variation in among-site effects and within-site effects, and the proportion of the surviving population sampled.

We also included a "schooling" parameter. Salmonids are a schooling species in general and survival of fish within a school may be correlated. When set to a value greater than one the "schooling" parameter allows the assumption of independence of fish to be violated. Using the model's output, one can go through a number of possible scenarios and determine which designs provide sufficient statistical power.

Workshop Discussion

Q. What would happen to your results if you [inaudible] interaction?
A. In this case we were creating our own reality, and we did not build term of interaction into the model. If we put interaction in there, it would depend on the severity of interaction, and would

tend to reduce power. We did not expect an interaction between our survival rates for the treatment groups relative to acclimation sites. There was not anything we saw that seemed to have the potential to create a large ……[inaudible].

Q. After hearing over the last couple of days about the differences in velocity, turbidity, temperature, predation effects, etc., at different release sites, has that caused you to rethink things a bit and consider the possibility that there may be some strong interactions?

A. That is possible. I think it may be time to revisit that issue.

<u>Presentation 4.2:</u>

Stealth and Visible Marks for Embryonic and Juvenile Salmonids

Steven L. Schroder, E.C. Volk, and Curtis M. Knudsen

Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, WA 98501-1091

Synopsis

To test the effects of various rearing tactics on the postrelease survival of juvenile salmonids, fish representing each treatment must receive marks or tags that can be used to identify their origins. There are a large array of potential marking methods available, each with its own benefits and costs. One of the most commonly used procedures is the Coded-Wire Tag and its accompanying adipose clip. CWTs can be applied at the juvenile stage in various body locations and retention is excellent. In some cases, however, it is not practical to use this tool, either because the fish are too small or physiologically fragile.

During the past decade we developed two marking methods that can be used to mark embryonic and juvenile salmonids without having to handle each fish individually. The first one, thermal marking, can be used to mark fish while they are in the eyed-egg and alevin stages. The marks are created by subjecting embryos to a predetermined set of abrupt thermal changes (usually a decrease of 40° C). Each time the a fish experiences such a decline, a pronounced proteinaceous band is induced into the micro-structure of its otolith. Initially we used multiple, evenly spaced bands to create recognizable marks, however, as our needs for multiple codes grew it became clear that a systematic rule for applying bands needed to be developed. We now use bar code symbols, called the 'two of five' rule, to create thousands of unique codes.

The other mass marking tool is simple immersion into solutions of alkaline earth salts. This technique can be used to mark fish at the moment of fertilization, after hatching, and anytime thereafter. Of the chloride salts we have tried, strontium chloride hexahydrate provided marks that were the most easily detected. Recently, we also evaluated two other methods that can be used to induce visible marks on juvenile salmonids. In one, microspheres of fluorescent material are injected via air pressure into fin rays. In this case each fish must be handled, but this procedure is quick and leaves a long-lasting mark that is readily detected. In the last procedure, a thin band of elastomer is injected into the clear adipose tissue located just posterior to the eye. When we evaluated this mark we noticed that the material tended to break apart and was completely shed in 20% of the fish we marked. New marking materials and methods of

application are being commercially developed for both the visible marks briefly described above and these tools promise to be valuable additions to the fish marking repertory.

Workshop Discussion
No discussion was recorded

<u>Presentation 4.3:</u>

The Introduction of Sockeye to Frazer Lake, Kodiak Island, Alaska

Carl Burger
U.S. Fish and Wildlife Service, 1440 Abernathy Rd., Longview, WA 98632

Synopsis
Not Submitted

Workshop Discussion
No discussion was recorded

SESSION V. MANAGEMENT CONSIDERATIONS

Session Moderator:
R.Z. Smith
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Panel:
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PANEL DISCUSSION

Moderator. (having first provided a general introduction to the session): I would now like to ask the panel to spend a few minutes talking about the next steps to take for Natural Rearing Systems. We have heard a lot of interesting presentations over the past two days, concerning techniques and concepts that could provide immediate benefit to many hatchery operations. But given the fact that our record of implementing hatchery advances is not all that good, how do we implement them? What do our panelists think we ought to do with this information?

Crateau. From my standpoint, that of a manager of a large mitigation program, while many of the conference presentations have been intriguing, the jury is still out on many of them in terms of their ultimate effect on adult returns. As a hatchery manager, I have to look at any innovation from a practical point of view: how much will it cost, and to what extent will it interfere with other hatchery operations? Some of the things we have been hearing about, such as providing cover and changing the background colors of the rearing tanks to encourage more natural coloration in the fish, seem quite practical. However, one of the things that struck me over the past two days is how difficult it is to measure the results of some of these experiments, given the large number of confounding mechanisms at work in the riverine and oceanic environments. My bottom line, as the manager of a large mitigation hatchery, is smolt-to-adult returns. Frankly, I am not sure it is possible to quantify the effects of some of the techniques we have been talking about at this workshop, as there are so many other factors that go to work after the fish are released. My inclination is to let some of this experimental work run a little longer, so that we can get a better picture of their effects on adult returns before making any major resource commitments at the production level.

Wright. I suggest that future natural rearing research focus not on areas that might be expected to provide only small biological gains, or that might be applicable only within a limited geographic area, but on areas that show the potential to produce large smolt-to-adult survival benefits over wide geographic areas; for example, in some of the terminal feeding and rearing strategies we have been discussing. Perhaps the time has come to design a model hatchery where natural rearing techniques can be tested on a production scale.

Berry. I agree with Ed (Crateau) that any significant changes to our hatchery operations need to be based on sound research. With funding becoming tighter and tighter for many hatchery programs, and budgetary considerations becoming more and more problematic, from a management perspective, increased competition for available funding means that any new programs or projects will need to have a very sound technical basis if they are going to be funded.

Burger. From my perspective, I am extremely encouraged by the research results presented at this workshop. Several papers in particular really provided me with a wealth of new information. However, I have to agree that the jury is still out, for many of the projects we have heard about over the past two days, on their ultimate effect on the bottom line: adult returns. Some of that data will be available in the next year or two. I think there are some mechanisms and factors that we clearly need to understand better before we can come to a consensus and change direction. However, I also agree that there a number of things that can done easily and inexpensively that will increase the overall quality of our fish.

Fuss. With the development of a Washington State wild salmonid policy, and with the Endangered Species Act bearing down on us, I suspect that hatchery programs will be changing in Washington. Most of those changes will be concentrated in our anadromous programs. Will artificial substrates and other NATURES-type rearing techniques be incorporated into Washington's anadromous fish hatchery programs? Probably, where applicable, but determining where they are applicable is not going to be easy. It has always been the philosophy of the Assessment and Development division of the State Hatcheries Program to produce the best-quality fish we can. Among other things, that means fish that survive well to adulthood, as well as hatchery fish that cause minimal disruption to naturally-spawning populations. The bottom line is that hatcheries are really nothing more than a tool, which can either be used correctly, or incorrectly. My hope is that, through the kinds of discussions we have had over the past two days, we can improve the effectiveness of that tool.

Wright. I am curious about the response of those in the audience who actually work at hatcheries to some of the ideas that have been put forward in the last two days. From a production standpoint, how practical really are things like camouflage covers, gravel substrates, and midwater debris?

Dorn. Actually, we have already been doing many of the things that have been mentioned, such as earthen ponds, low densities, plenty of cover, and volitional release, etc., with fall chinook and chum salmon at our facility. The fish are handled very minimally. I would add that I like a lot of the ideas I have heard over the past two days, and will be giving some thought to how to incorporate some of them into our operation. The bottom line, from my standpoint, is that we need to be successful. If we are going to continue to see salmon in our local area, I am convinced that hatcheries are going to play a critical role. I would add that one thing that has not been touched on so far in this conference is the importance of protecting the few productive watersheds that remain from human encroachment. That is a problem that is especially acute in the Puget Sound area.

Crateau. My agency intends to make the jump from an experimental level to a production level with some of the techniques that have been discussed at this conference, such as natural substrate, camouflage covers, in-stream structures, higher flow rates, etc., at Lookingglass Hatchery. We want to look at the practicality of these techniques in a large-scale production setting, and to evaluate things like potential disease problems, cleaning, etc. I would add, however, that until we make some significant changes to the hydro-system, I am not sure that anything we do at the hatchery level will produce the kinds of survival gains we would all like to see. But I do think it makes sense to try to mimic nature to the greatest extent possible.

Anon. As the former manager of a large hatchery facility, I think a lot of the ideas that have been put forward over the past couple of days are implementable. Hatchery managers will do almost anything you ask of them, as long as you can show them the benefits of these innovations. Particularly for things that are relatively simple and inexpensive to implement. If you can get buy-in from hatchery personnel and managers, they will make them work.

Anon. As another former manager, one of the most useful things I heard yesterday was the night release scenario. I think that makes a world of sense from the standpoint of reduced predation, and it is something that is both inexpensive and simple to implement.

Burger. From the standpoint of selling these ideas to the hatchery managers, are there plans to produce a written summary of the proceedings of the last couple of days? Because I think that would be a very useful sales tool.

Flagg. Our intent is to take Synopses from each of the talks, and to package those with written notes from the panel discussions and question-and-answer sessions that have occurred during the conference. That document will then be distributed to all the workshop participants.

Anon. Another useful tool would be to put some of these NATURES projects on video.